

HELSINGIN YLIOPISTON KOTIELÄINTIETEEN LAITOKSEN JULKAISUJA
UNIVERSITY OF HELSINKI, DEPT. OF ANIMAL SCIENCE, PUBLICATIONS

54

Influence of the timing of the harvest
of primary grass growth on herbage quality
and subsequent digestion and performance
in the ruminant animal

Marketta Rinne

Academic dissertation

To be presented, with the permission of
the Faculty of Agriculture and Forestry of the University of Helsinki,
for public criticism in Auditorium B2, Viikki,
on December 21st 2000, at 12 o'clock noon

Helsinki 2000

Custos

Professor Liisa Syrjälä-Qvist
University of Helsinki, Finland

Supervisors

Professor Pekka Huhtanen
Agricultural Research Centre, Animal Production Research, Finland

Dr. Seija Jaakkola
Agricultural Research Centre, Animal Production Research, Finland

Reviewers

Dr. Oiva Niemeläinen
Agricultural Research Centre, Plant Production Research, Finland

Docent Jouko Setälä
Association of Rural Advisory Centres, Finland

Opponent

Professor Cledwyn Thomas
Scottish Agricultural College, Food Systems Division, Scotland, UK

ISBN 951-45-9646-3
ISBN 952-91-3015-5 (pdf)
ISSN 1236-9837

Electronic publication available at
<http://ethesis.helsinki.fi>

Helsinki University Press
Helsinki 2000

Rinne, M. 2000. Influence of the timing of the harvest of primary grass growth on herbage quality and subsequent digestion and performance in the ruminant animal. University of Helsinki, Department of Animal Science. Publications 54. 42 p. + 5 encl.

Abstract

This thesis consists of experiments in which the timing of harvest in the primary growth of grass was used to manipulate forage quality. The objectives of this work were to qualify and quantify the effects of timing of harvest of temperate forage grass species in primary growth on ruminant digestion and production. The emphasis was placed on the nutritional quality of forages, ability of different experimental techniques and measurements to detect differences between forages, effects on ruminant digestive processes, feed intake, and finally milk production with special reference to the interactions between forage quality and concentrate supplementation.

In the first experiment (publications I and II) four silages were harvested at one week intervals from a primary growth of timothy-meadow fescue. The D-value of ensiled herbage decreased in a curvi-linear manner with advances in herbage maturity (750, 746, 681 and 654 g/kg DM in the order of harvest date). The silages were fed to four ruminally and duodenally cannulated young cattle at a restricted level of feed intake to allow comparisons at equal levels of DM intake. Ruminal pH was lower, ammonia concentration higher, and the molar proportion of acetate increased, propionate remained stable and butyrate decreased with increasing maturity of ensiled grass. The markedly higher intake of N when earlier cut silages were fed resulted in only minor and non-significant increases in the flow of non-ammonia-N into the duodenum. The experiment included comparisons of methods for the measurement of digesta kinetics and although remarkable between-method differences were observed, they all revealed that changes in forage quality clearly affected digestive processes. The rate of NDF digestion decreased while the rate of passage increased with a concomitant increase in the pool size of NDF in the rumen.

In the second experiment (publication III), four silages were produced with D-values of 739, 730, 707 and 639 g/kg DM in the order of harvest date, and fed *ad libitum* to ruminally fistulated dairy cows. Again differences similar to those in the first experiment were found in rumen fermentation parameters, rates of NDF digestion, passage and rumen fill. These responses were also reflected in feed intake and milk production even though cows were in late lactation. The observation that increased feed intake of the early-cut silages was accompanied by decreased rumen fill suggests that the regulation of feed intake was not exclusively related to this parameter.

The same feeds as used in experiment 2 were used in the third experiment (publication IV) using 32 intact dairy cows. The cows increased their energy corrected milk production by 0.50 kg and silage DM intake by 0.162 kg per 10 g/kg DM increase in D-value. The silages were supplemented with 7 or 10 kg of concentrate which contained 0 or 1.15 kg rapeseed meal. No interactions between silage harvest date and concentrate supplementation were found revealing that the possibility to compensate for poor silage quality with concentrate feeding is limited.

In the fourth experiment (publication V) the effects of the timing of primary growth harvest on the yield and quality of organically grown mixed leys was studied both in primary growth and subsequent regrowth over two years. Delaying the harvest of the primary growth increased DM yield but decreased herbage digestibility. Reciprocal effects in the regrowth partly compensated for these changes but the pattern found in primary growth was still discernible in weighted herbage yields over both harvests.

In conclusion, timing of the harvesting of primary growth clearly affected forage quality and subsequently digestion and animal performance of ruminants. Rapid but sometimes markedly curvi-linear decrease in forage D-value with advances in primary grass growth emphasizes the importance of the correct harvesting date. The impact of different harvesting strategies across the entire growing season remains unclear, and further research is required.

Acknowledgements

I wish to thank prof. Liisa Syrjälä-Qvist at the Department of Animal Science, University of Helsinki, and prof. Tuomo Varvikko at the Animal Production Research, Agricultural Research Centre (MTT), for giving me the opportunity to conduct the work reported in the thesis at these institutions.

The role of my supervisors, prof. Pekka Huhtanen and Dr. Seija Jaakkola has been invaluable during my studies. I especially wish to thank Pekka for making me understand the importance of the quantitative approach into ruminant nutrition. Seija encouraged me with her own positive example to choose research as a career.

I am also indebted to the Faculty nominated referees, Dr. Oiva Niemeläinen and docent Jouko Setälä for valuable comments in improving the manuscript of this thesis.

The work in the fields, barns and laboratories would never have been successful without the input of numerous staff members both at the University and MTT. You are too many to be named but I greatly appreciate the contribution of you all. I want to extend my warmest thanks to the whole scientific community I have been working with, including the IP group.

I also want to acknowledge the role of the cattle in conducting this work. Working with you, and for you, has been an important source of motivation for me. I want to mention especially the gentle bull H.H. Tricót in Exp. 1, the sympathetic fistulated cow Muru in Exp. 3 and Vaisu, the "you don't take blood samples from me" cow in Exp. 4. The sweet digestibility trial sheep easily charmed me.

The financial support from the Agricultural Research Foundation of August Johannes and Aino Tiura, Kemira Foundation, Finnish Konkordia Foundation and Finnish Association of Academic Agronomists is gratefully acknowledged.

My best thanks go to Dr. Kevin Shingfield for excellent linguistic revision of the thesis and for being a good colleague for many years.

Finally I wish to thank my dear husband Reijo. You may not always have been so patient when I brought the papers with me home. But you have proven to me that there are other important things in life besides the scientific work. I thank you for this lesson from the bottom of my heart.

In Vivola, November 2000

Marketta Rinne

Influence of the timing of the harvest of primary grass growth on herbage quality and subsequent digestion and performance in the ruminant animal

List of original publications

This thesis is based on the following original publications subsequently referred to in the text by their Roman numerals:

- I **Rinne, M., Jaakkola, S. & Huhtanen, P.** 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. *Animal Feed Science and Technology* **67**: 1-17.
- II **Rinne, M., Huhtanen, P. & Jaakkola, S.** 1997. Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. *Animal Feed Science and Technology* **67**: 19-35.
- III **Rinne, M., Huhtanen, P. & Jaakkola, S.** 2000. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. *Manuscript*.
- IV **Rinne, M., Jaakkola, S., Kaustell, K., Heikkilä, T. & Huhtanen, P.** 1999. Silages harvested at different stages of grass growth v. concentrate foods as energy and protein sources in milk production. *Animal Science* **69**: 251-263.
- V **Rinne, M. & Nykänen, A.** 2000. Timing of primary growth harvest affects the yield and nutritive value of timothy-red clover mixtures. *Agricultural and Food Science in Finland* **9**: 121-134.

The above mentioned articles were reprinted with the kind permission of copyright owners Elsevier Science, British Society of Animal Science and Agricultural and Food Science in Finland.

The first experiment (publications I and II) was conducted within the Department of Animal Science, University of Helsinki. The second (publication III) and third (publication IV) experiments were carried out at the Animal Production Research, Agricultural Research Centre (MTT) in Jokioinen. The field work of experiment 4 (publication V) was conducted within Ecological Production, Resource Management Research Unit of MTT in Juva, and the samples were analysed at the laboratory of Animal Production Research, MTT, Jokioinen.

The author participated in conducting the experiment and calculating the results reported in publications I and II, and was the main author of the published material. The author participated in planning and conducting the experiments and took full responsibility for the calculation and reporting of experimental data documented in publications III, IV and V.

Abbreviations

AA	Amino acid
AAT	Amino acids absorbed from the small intestine
ADF	Acid detergent fibre
CP	Crude protein
DCP	Digestible crude protein
DM	Dry matter
DMI	Dry matter intake
D-value	Digestible organic matter in the dry matter
ECM	Energy corrected milk
EP	Escapable pool
FI	Feed intake
INDF	Indigestible neutral detergent fibre
k_d	Rate of digestion
k_p	Rate of passage
LW	Live weight
ME	Metabolizable energy
MPS	Microbial protein synthesis
N	Nitrogen
NIRS	Near infrared reflectance spectroscopy
NDF	Neutral detergent fibre
NEP	Non-escapable pool
OM	Organic matter
PBV	Protein balance in the rumen
SDMI	Silage dry matter intake
VFA	Volatile fatty acid
WSC	Water soluble carbohydrates

Contents

Abstract

Acknowledgements

List of original publications

Abbreviations

1	Introduction.....	1
2	Material and methods.....	2
2.1	Description of experiments.....	2
2.2	Production of experimental feeds	3
2.3	Experimental procedures.....	3
3	Results and general discussion.....	4
3.1	Effects of the timing of harvest on forage quality	4
3.1.1	Development of grass in primary growth.....	4
3.1.2	Sward development over the whole growing season.....	7
3.1.3	Silage preservation.....	8
3.2	Description of forage quality	10
3.2.1	Digestibility.....	10
3.2.1.1	Chemical composition as a predictor of forage digestibility	10
3.2.1.2	Assessment of digestibility	12
3.2.2	Protein value	14
3.3	Physiological responses of ruminants to forage quality.....	18
3.3.1	Rumen fermentation.....	18
3.3.2	Rumen fill.....	20
3.3.3	Digestion kinetics	20
3.3.4	Feed intake	23
3.4	Effects of the timing of forage harvest on milk production.....	25
3.4.1	Responses to silage digestibility	25
3.4.2	Interactions between silage D-value and concentrate supplementation ...	29
3.4.3	Interactions between silage D-value and protein supplementation	32
4	Conclusions and suggested future research	34
	References	36

Publications I-V

1 INTRODUCTION

The quality of forages manipulated by the stage of grass growth has been intensively studied throughout the history of animal science because of its importance to the biological and economic performance of ruminant based production systems. It is beyond the scope of this thesis to review all previous literature. The thorough investigations of Homb (1953) and Kivimäe (1959) show that the interest to improve the use of home-grown feeds was compelling after World War II.

Later the so called "Green Line" was implemented in Finland. Concomitant with the development of efficient ensilage techniques, the harvesting of grass at an early stage combined with relatively high levels of nitrogen (N) fertilization was recommended. The goal was to produce feeds with a high digestible crude protein (DCP) concentration and the value of grass as a source of on-farm protein for cattle was emphasized (Lampila and Ettala 1971, Salo 1978). This was done despite no benefits in milk production being observed from increased crude protein (CP) concentration through N fertilization (Huokuna 1968, Ettala et al. 1971, 1974). Experiments where the true value of forage protein could have been evaluated by e.g. recording responses to protein supplementation were not conducted at that time. Earlier harvest of grass elicited higher milk production responses (Ettala et al. 1978), but later work supported by the adoption of a new feed evaluation system in 1995 in Finland (Madsen et al. 1995, Tuori et al. 1998, 2000) has revealed that this was due to increases in energy rather than DCP intake of the cows.

The unique digestive system of ruminants including pregastric microbial fermentation of feeds has allowed them to survive and thrive on diets based mainly on plant cell walls indigestible to mammalian digestive enzymes (Van Soest 1994). The composition of feeds consumed by ruminants differs markedly from the nutrients available to the metabolism of the ruminant due to the modifications caused by microbial fermentation in the rumen. To be able to predict the supply of energy and other nutrients to ruminants, processes occurring in the rumen have to be assessed.

Also aspects related to the control of feed intake (FI) have features different from those in monogastric animals. Intake of energy has generally been accepted as the most important constraint limiting ruminant production (Mertens 1994, Van Soest 1994) even though energy and protein supplies are difficult to separate in ruminant nutrition. The energy supply to an animal is controlled by the amount of feed offered to the animal, the amount of feed the animal consumes and the concentration of available energy in a unit of feed, i.e. the digestibility. Digestibility of grass is to a large extent determined by the stage of growth of the plants. Although variation is greatest in FI, the digestibility of the forage also affects FI thus increasing its significance in ruminant nutrition.

In 1999, approximately 60 % of feed energy consumed by dairy cows in Finland originated from forages including silage, hay and pasture. Silage alone provided slightly less than 40 % of feed energy for dairy cows (Maaseutukeskusten liitto 2000). Temperate grasses are higher in digestibility than tropical grasses (Van Soest et al. 1978), which gives the Finnish animal production sector a great opportunity to utilize efficiently forage based diets. However, the decline of forage digestibility is fastened the further north the plant is growing (Deinum et al. 1981) emphasizing the critical importance of harvesting date. In spite of its great significance, few practical advisory services assisting the timing of forage harvesting have been established. Many methods have been proposed to be useful in this sense including herbage chemical composition or plant phenological indexes, and environmental parameters (Fick et al. 1994).

This thesis consists of experiments, in which the timing of harvest in the primary growth of grass was used to manipulate the quality of forage. The objectives of this study were to qualify and quantify the effects of timing of harvest of temperate forage grasses (mainly timothy and meadow fescue) in primary growth on

- * herbage production, with specific emphasis on nutritive value
- * ability of different experimental techniques and measurements to detect differences between forages
- * ruminant digestive processes and feed intake
- * milk production with a particular reference to the interactions between forage quality and concentrate supplementation.

2 MATERIAL AND METHODS

2.1 Description of experiments

The common aspect in the experiments currently documented was that the quality of grass was manipulated by the timing of harvest of the primary growth. In other aspects, the primary objectives as well as the methodology used differed markedly (Table 1).

Table 1. Description of experiments.

Art.	Exp.	Animals (n)	Feed set (no. of harvests)	Feeding	Areas of study
I	1	Fistulated young cattle (4)	1 (4)	Restr.	Rumen fermentation and protein utilization
II					Rumen fill and digesta kinetics
III	2	Fistulated dairy cows (4)	2 (4)	Ad lib.	Rumen fill, digesta kinetics and digesta particle size
IV	3	Intact dairy cows (32)		Ad lib.	Feed intake, milk production and interactions with concentrate feeding
V	4	---	3 (3) Two years	---	Forage yield, chemical, botanical and morphological composition of primary growth and subseq. regrowth

The first experiment (I, II) focused on rumen metabolism and it was conducted in young cattle with restricted FI to allow comparisons to be made at an equal level of DM intake (DMI). In the second experiment (III), rumen metabolism of dairy cows fed *ad libitum* was studied. The third experiment was a milk production trial, where different concentrate supplementation treatments were also examined (IV). The fourth experiment (V) concentrated on forage production over two years and measurements were also made from the subsequent regrowth.

2.2 Production of experimental feeds

In experiment 1, a mixed ley consisting primarily of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) was harvested on four occasions at approximately one week intervals from the same field at the Viikki experimental farm, University of Helsinki, Finland (60°N). The second set of silages was also prepared from a timothy-meadow fescue grass and harvested according to a similar schedule but at the Lintupaju experimental farm, Agricultural Research Centre, Jokioinen, Finland (61°N). These silages were utilized in experiments 2 and 3. For both sets of silages, grass was ensiled directly with 4 l formic acid as a preservative. The first set of silages was ensiled into cylindrical 3 m³ glass fibre silos (3 silos per each feed). The second set of silages was ensiled into 70 t bunker silos.

In the fourth experiment, organic leys comprising of timothy, meadow fescue and red clover (*Trifolium pratense*) grown at Ecological Production, Resource Management Research, Agricultural Research Centre, Juva, Finland (62°N) were used. The partition of growth time between primary growth and regrowth was studied in a two-cut system over two years. In both years, the primary growth was harvested on three different occasions. All subsequent regrowth areas were harvested on the same day in the autumn. No feeds were prepared for animal experiments, but herbage samples were collected for chemical analysis.

2.3 Experimental procedures

This chapter gives a brief overview of the experiments. Due to a wide variety of experimental procedures used in the individual experiments, they are not reviewed in detail, but are fully documented in publications I-V.

Four ruminally and duodenally cannulated animals were used in experiments 1 (young cattle) and 2 (lactating dairy cows). The experiments were conducted as balanced 4H4 Latin square designs. The FI of young cattle was restricted to 70 g DM/LW^{0.75} with proportionately 0.3 of their diet fed as concentrate. In the end of each experimental period, a 3-day *ad libitum* silage feeding period was included. Dairy cows were fed *ad libitum* and concentrate comprised on average proportionately (on a DM basis) 0.34 of their diet. Moderate concentrate supplementation was chosen so that differences between the silages would be discernible and that the experimental observations would be comparable to practical feeding situations.

Rumen fermentation was measured in both experiments by sampling rumen fluid through the rumen cannulae at regular intervals. Rumen evacuations were conducted in both experiments to estimate rumen pool size and digesta kinetics, and nylon bag incubations were used to determine the rate of neutral detergent fibre (NDF) degradation and potential NDF digestibility.

Microbial protein synthesis (MPS), duodenal nutrient flow and digesta kinetics using marker methods were determined in Exp. 1. In Exp. 2, the particle size distribution of feeds, rumen digesta and faeces was determined by wet sieving. The *in vivo* digestibility of silage sets 1 and 2 was determined using cattle [total faecal collection in Exp. 1 and spot sampling of faeces using acid insoluble ash as an internal marker in Exp. 2] and mature wether sheep (total faecal collection, feeds fed at maintenance). The D-values based on the digestibility measurements in sheep have been used in the figures presented later in this thesis. For silage set 2, MPS in the rumen of sheep was estimated from urinary purine derivative excretion.

In Exp. 3, 32 intact dairy cows were fed *ad libitum* the same silages as in Exp. 2 supplemented with either 7 or 10 kg concentrate, that contained either 0 or 1.15 kg rapeseed meal. The amount of CP originating from rapeseed meal was planned to correspond to differences in CP intake predicted for *ad libitum* intakes of silages harvested at one week intervals. The 16 diets were fed to the cows in a cyclic change-over design with 4 periods. Experimental measurements included FI, milk production, blood parameters, *in vivo* digestibility (based on spot sampling of faeces and acid insoluble ash as an internal marker) and feeding behaviour.

The fourth experiment involved sampling of organically grown leys both in primary growth and subsequent regrowth. The botanical composition of leys was determined as well as the morphological composition of the main species, timothy and red clover. Leaves and stems were further evaluated in terms of chemical composition and *in vitro* digestibility. The samples from the first year of the study were also submitted for *in vitro* gas production measurements in order to assess rate of digestion.

3 RESULTS AND GENERAL DISCUSSION

3.1 Effects of the timing of harvest on forage quality

3.1.1 Development of grass in primary growth

The development of digestibility and changes in the chemical composition with progressing primary growth of grass are well documented in numerous experiments (refer to Van Soest et al. 1978, Thomas and Thomas 1985, Van Soest 1994, Beever et al. 2000). The primary growth of grass is characterized by stem elongation as tillers produce seedheads. The changes observed in the nutritional quality of the plants are partly caused by changes occurring within the stems and the leaves, and partly by the rapidly increasing proportion of stems, which at the time of harvest are nutritionally less valuable than leaves (Terry and Tilley 1964, Salo et al. 1975, V).

The trends as functions of growth time observed in the present material are presented in Figure 1. The average daily decline in the digestible organic matter (OM) concentration in DM (D-value) was 3.9, 4.5, 5.7 and 5.6 g/kg in the gramineous samples in I, III, V (1995) and V (1996), respectively. The general trends correspond well to earlier reported values (e.g. Table 2). In addition to the decline in D-value, other typical trends associated with progressing maturity included the decrease in grass CP content while concentrations of cell wall components [NDF, acid detergent fibre (ADF) and lignin] increased (Figure 1).

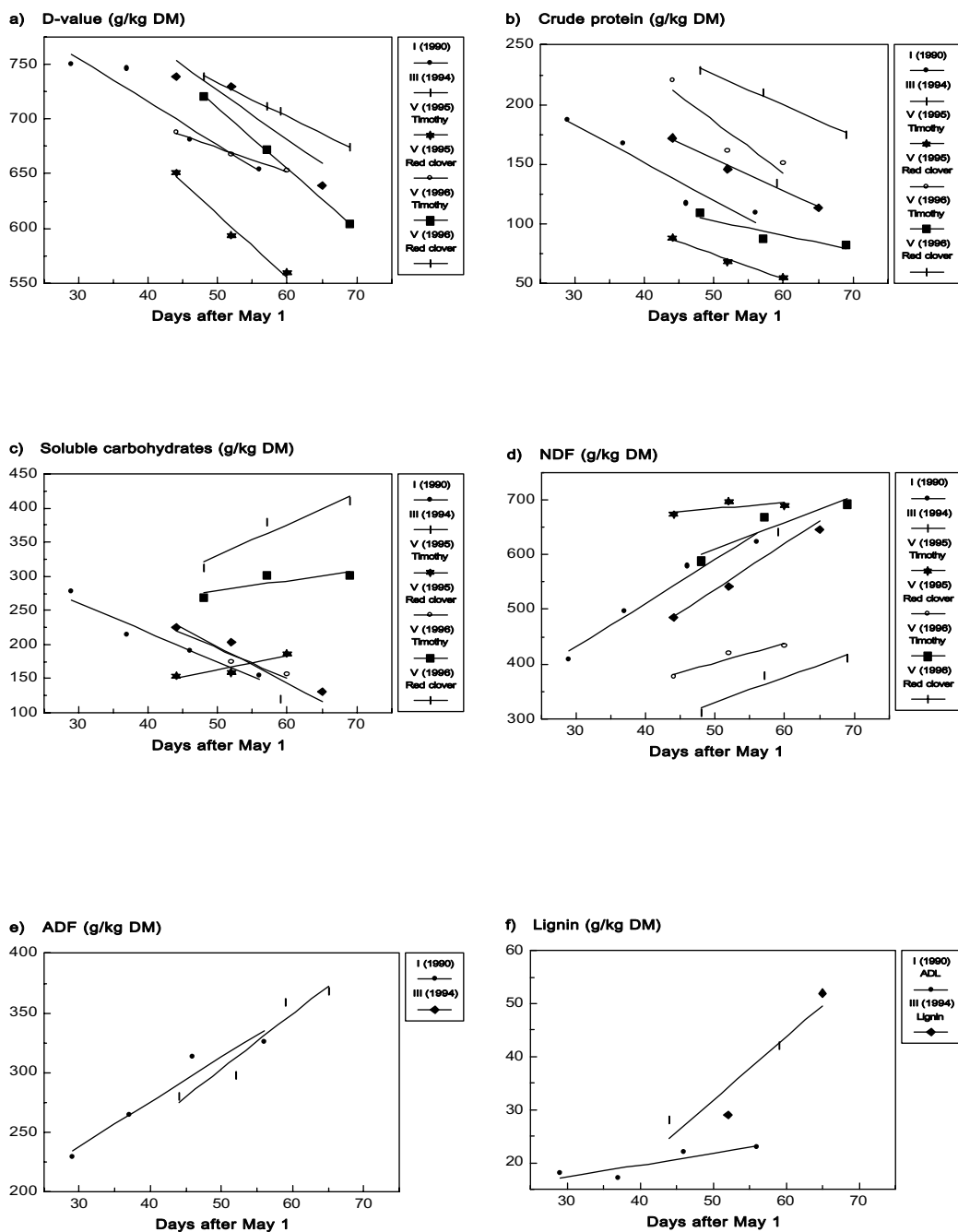


Figure 1. Development of forage D-value (a) and concentrations of crude protein (b), soluble carbohydrates (calculated as DM - ash - CP - NDF - estimated crude fat; c), NDF (d), ADF (e) and lignin (f) in relation to growth time.

The clear curvi-linear development of D-value in silage sets 1 and 2 deserves further attention. In 1, the greatest difference in the quality was found between feeds II and III, and in 2 the differences between silages I to III were relatively minor compared to IV. The need to accurately determine the date of harvest is emphasized in situations where grass development is curvi-linear in relation to time. The curvi-linear development and variation between different years can be decreased by relating grass development to environmental factors such as cumulative temperature, at least under Nordic climatic conditions (Pulli 1980a,b, Huokuna and Hakkola 1984, Thorvaldsson and Fagerberg 1988, Gustavsson 1994, Rinne et al. 1999a, V).

In Finland, grass samples are annually collected around the country by Valio Ltd. and analysed for CP and crude fibre concentrations. Since 1998 D-value has been included to assist farmers in the correct timing of silage harvest. The findings of the present experiments inspired further work for the development of an advisory service for Finnish farmers. During summer 2000, estimates of D-value in primary growth based on cumulative temperature and geographical location within Finland were provided via the Internet (Artturi 2000, Rinne et al. 2000b).

The curvi-linear development of grass may sometimes lead to misleading conclusions from individual experiments, particularly if the time span used to monitor grass development is very short. E.g. in forage set 2, if only first 3 silages had been harvested, the conclusions of the experiment had been different than when the fourth harvest was also included.

The daily D-value decline in gramineous samples was steeper than that in red clover (V). Similar trends have been found in other experiments (Table 2) showing that the rate of daily D-value decline in red clover was only half of that in grasses. Furthermore, the production potential of red clover may be better than that of grass based forages at the same digestibility (for further discussion, refer to IV). Thus the contribution of leguminous forage plants in mixed leys must be taken into account, when selecting the appropriate harvesting strategy. Furthermore, within temperate gramineous species, between-species and between-variety differences have been observed (Davies 1976, Åman and Lindgren 1983, Cherney et al. 1993, Nissinen and Hakkola 1995, Bélanger and McQueen 1996, 1998b, Pärssinen 1999). However, changes associated with increases in maturity generally exceed between and within species variations.

Table 2. Comparison of the decline in daily D-value of forage grasses and red clover (g/kg DM) during primary growth.

Reference	Forage grasses	Red clover
Fagerberg and Ekbohm (1995)	3.1	1.9
Hakkola and Nykänen-Kurki (1994)	5.1	1.7
Nykänen et al. (2000)	5.3	3.0
Salo et al. (1975)	6.0	3.3
V	5.6	2.6
Mean	5.0	2.5

Herbage growth creates yield such that the opposing development of forage quality and quantity is the critical factor which makes the timing of harvest date so important. From a nutritional point of view, DM yield is clearly an inadequate measure of herbage output. The yield of digestible OM (DM yield \times D-value) may also be unsuitable even though it is better than simply DM yield alone. High digestible OM yields may be obtained in situations when the nutritional quality of the herbage is very low but DM yield is great. In such cases, it may be difficult to formulate adequate diets for dairy cows. High level of concentrate supplementation would be needed to sustain milk production,

but this strategy will result in reduced silage intake.

Herbage yield was unfortunately documented in only one of the experiments (V). In the primary growth, these organically maintained plots generated daily on average 116 kg DM per hectare. In a later experiment (Rinne et al. 2000a), conventional timothy-meadow fescue leys similar to those used in silage sets 1 and 2 have accumulated on average 146 kg DM per day in primary growth.

3.1.2 Sward development over the whole growing season

In the experiments reported in this thesis, only one (V) also considered grass regrowth. The same trend can be found in the literature with the majority of experiments concentrating on primary growth. This is understandable, because the generative growth pattern in primary growth is likely to cause greater differences in feed quality than the less profound changes in the vegetative regrowth. Furthermore, the numerous options created by different harvesting times in primary growth, different lengths of regrowth periods, and different numbers of regrowth cycles, result in experimentation which tends to become too massive and complicated to be adequately completed.

However, from the practical viewpoint, the quality and quantity of feeds harvested over the whole growing season form the basis for winter feeding, and should be considered. In V, earlier harvest of primary growth had only a marginal effect (-95 kg) on the digestible OM yield harvested over the entire summer, but the average forage D-value was higher (690 vs. 631 g/kg DM). Syrjälä and Ojala (1978) and Frame (1987) as well as further experimentation conducted at MTT (Rinne et al. 2000a), confirm that the changes in the quality and quantity of regrowth are opposite to those found in primary growth. The differences in the weighted values across all harvests both in terms of DM yield and D-value were smaller than those observed for either harvest viewed separately, but the pattern observed in the primary growth tended to be discernible also in the weighted total yield.

There remains several uncertainties concerning the optimal harvesting strategy over the whole growing season. For example, the timing of regrowth was not studied in the current experiments. Van Soest et al. (1978) reported that the digestibility of temperate regrowth may even increase with progressing growth in the autumn. Similar results were also obtained in Finland (Rinne et al. 2000a), although often a slight decline in forage quality is found also in regrowth (Syrjälä et al. 1978, Åman and Lindberg 1983, Thorvaldsson and Andersson 1986, Thorvaldsson and Fagerberg 1988, Fagerberg and Ekbohm 1995). The different development of regrowth digestibility compared to that in primary growth may be caused by the lower growth temperatures retarding lignin synthesis (Van Soest et al. 1978) and differences in the growth pattern, since the proportion of leaves in the total herbage mass did not decline with advances in grass growth during autumn (Åman and Lindgren 1983, Rinne et al. 2000a).

There are some indications that the production potential of silage made from regrowth may not be as high as that of primary growth at the same digestibility (Heikkilä et al. 1998). Such comparisons have been scarce reported in the literature. Production responses to regrowths varying in digestibility are also poorly documented. The role of, for example, plant diseases which may invade the grass in the autumn on FI and nutritional quality are not known at least under Finnish conditions. Analytical problems may also be involved as preliminary results (Rinne et al. 2000, unpublished) suggest that the digestibility of regrowth may be overestimated when *in vitro* methods such as that of Friedel (1990) are used. There remains important questions to be solved before forage production and utilization of different forage batches over the whole growing season can be optimized.

3.1.3 Silage preservation

The systematic changes in herbage composition with progressing primary growth should also be considered from the viewpoint of forage conservation. Successful conservation is a prerequisite for efficient use of forages in many ruminant based production systems. The parameters in the raw material which are generally considered advantageous for good preservation include neither low nor very high DM concentration, low CP concentration and buffering capacity and a high water soluble carbohydrate (WSC) concentration (McDonald et al. 1991). However, all these parameters change systematically with progressing growth of grass suggesting that mature grass may be more suitable for ensiling. Paradoxically, strictly anaerobic conditions may be easier to achieve when grass is harvested at an earlier stage of growth.

The WSC concentration of grasses is essentially a function between photosynthetic production, maintenance, growth and storage requirements, and varies within a day, and in response to environmental conditions. In schematic presentations of grass development, the concentration of WSC is assumed to increase with progressing growth of grass (Beever et al. 2000). In Figure 2, WSC concentration in relation to D-value is presented in I and IV and in recent material from MTT (Rinne et al. 2000, unpublished). There was a tendency for higher WSC concentrations in immature grass, although the variation in these measurements was large (Figure 2a). For silages no grass maturity trends could be identified (Figure 2b) probably because of the greater conversion of WSC to fermentation acids in early cut silages. The postulated increase in WSC concentration of herbage with progressing growth does not seem to be very clear based on the current data consistent with that of Cone et al. (1999) and Keady et al. (2000).

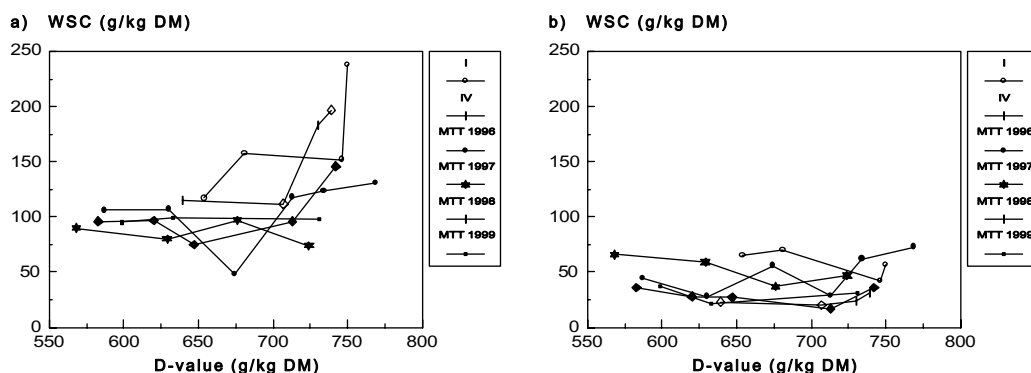


Figure 2. Water soluble carbohydrate concentration of grass (a) and silage (b) in relation to D-value.

Although the systematic changes in the chemical composition of herbage could be anticipated to risk the fermentation quality of early cut silage, this has not been observed either in experimental material (I, IV, Figure 3 based on the literature review data presented in Table 3) or in practice (J. Nousiainen, Valio Ltd., personal communication). In I and IV the concentration of fermentation acids was greater in silages harvested at an earlier stage of growth, but indications of poor preservation such as high pH, a large proportion of total N as $\text{NH}_3\text{-N}$, or marked increases in butyric acid concentrations were not found.

Differences in silage fermentation quality may occasionally be found, and they could, depending on the extent of the differences, either over- or underestimate the influence of variations in grass maturity on their intake potential. Based on a large data set, the concentration of total acids and the proportion of $\text{NH}_3\text{-N}$ in total N can be used to predict silage intake potential (Huhtanen et al. 2000b). Direct cutting and high level of formic acid were used in the present experimental silage sets to avoid possible confounding effects of different weather conditions during wilting and variations in herbage ensiling characteristics.

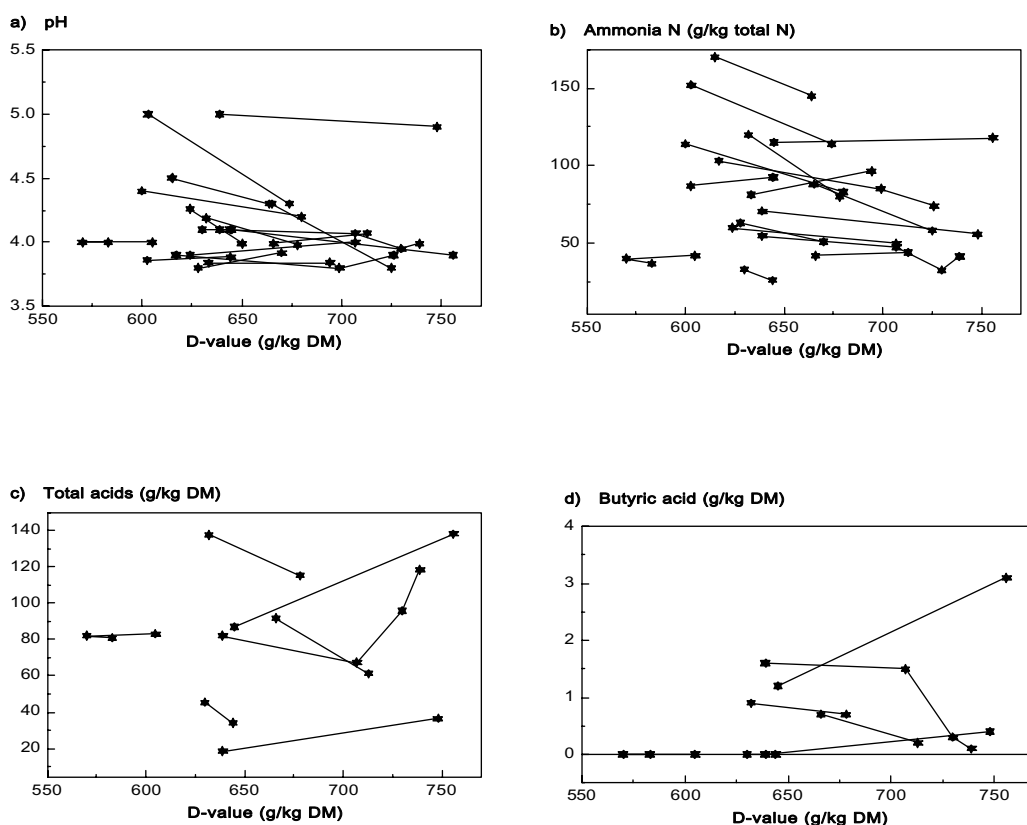


Figure 3. Silage fermentation quality expressed as pH (a), proportion of ammonia N in total N (b), concentration of total fermentation acids (c) and butyric acid content (d) in relation to D-value (data from experiments documented in Table 4).

3.2 Description of forage quality

3.2.1 Digestibility

The energy value of grass is primarily dependent on digestibility. According to the Finnish feed evaluation system, the metabolizable energy (ME) concentration (MJ/kg DM) of silage is calculated as $0.016 \times \text{D-value}$ (Tuori et al. 2000). Digestibility of a feed is not a chemical entity which could be analysed from the feed as such, but it represents the proportion of the feed energy/nutrients available to the animal during digestion. Digestibility of a feed not only depends on its intrinsic properties alone, but also on the animal consuming it and on other dietary constituents. Thus obtaining a reliable assessment of digestibility is problematic.

The major problem in interpreting published results reporting responses to forage quality is the lack of a uniform description of evaluated material. In some reports, no indications of forage digestibility are given, and when digestibility is measured, differences in methodology lead to estimates being associated with large variances. Reasonable comparisons between experiments are possible, if the forage digestibility is measured e.g. in digestibility trials with sheep or with universally common *in vitro* methods. If *in vivo* digestibilities from dairy cows consuming mixed diets are reported, the values are not easily comparable with data from other experiments.

3.2.1.1 Chemical composition as a predictor of forage digestibility

Chemical composition of forages is often used as an indication of digestibility. Unfortunately the relationships between CP and NDF, the common measured chemical components, with D-value is unclear (Figure 4).

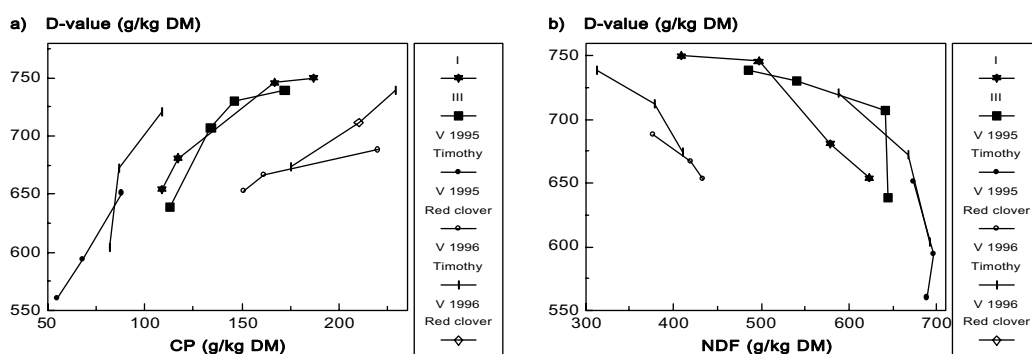


Figure 4. Prediction of forage D-value based on CP (a) and NDF (b) concentrations.

With respect to CP and NDF, red clover clearly deviates from grasses (Figure 4), but even within grasses, considerable problems remain. For CP, within each sample set, a decline in the concentration with progressing growth is evident. The level of CP in conventionally grown silages in I and III was higher than that of organically grown samples in V. This demonstrates the most important weakness of CP as an absolute predictor of D-value, i.e. its clear dependence on soil N availability. Grass plants take up the bulk of the N early in the growing season resulting in high CP

concentrations in immature plants. With progressing growth, the CP pool is diluted into a greater herbage DM mass resulting in decreased CP concentration (Thorvaldsson and Andersson 1986). The relationship between CP concentration and D-value may be strong within a particular ley on a single year, but the connection may be coincidental since D-value is determined by the stage of plant growth.

This view is supported by the fact that when herbage CP content is manipulated by N fertilization, the effects on herbage D-value are negligible (Thorvaldsson and Andersson 1986 including a literature review of 10 references, Frame 1987, Lindgren and Lindberg 1988, Keady et al. 1995, Bélanger and McQueen 1998a, Figure 5). Adequate N supply still remains an important factor in forage production because of the substantial increases in DM yield it elicits.

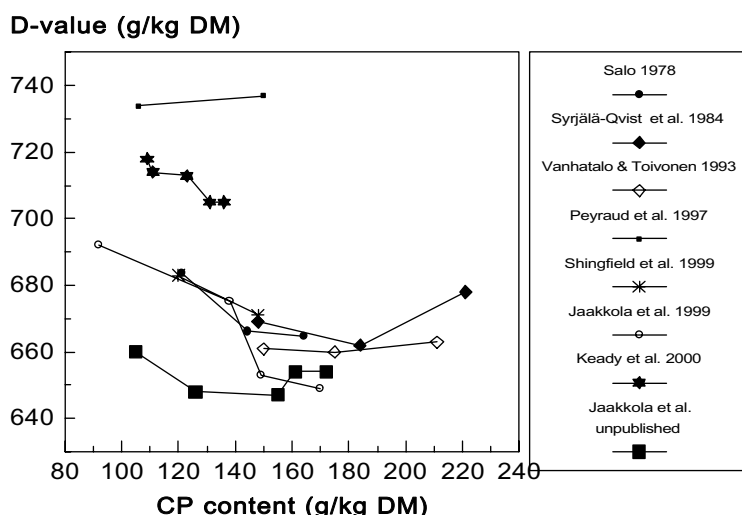


Figure 5. Relationship between CP concentration and D-value of silage for experiments that manipulated N fertilization level. The feeds were harvested on the same day.

The cell contents (DM-NDF) are considered to be completely digestible (Van Soest 1967) while cell walls (NDF) are not. Thus an increase in the NDF concentration of a forage generally results in a reduction of DM digestibility. However, the digestibility of NDF is highly variable, which reduces the usefulness of NDF concentration as a predictor of the digestibility of grass (Figure 4b). In II, the range in NDF digestibility was from 686 to 757 g/kg and in IV from 695 to 803 g/kg. The evaluation of 7 different fibre fractions (lignin, cellulose, crude fibre, hemicellulose, ADF, NDF and modified ADF) revealed that none of them allowed an accurate prediction of OM digestibility (accountable variance 0.22-0.56) while an *in vitro* based measurement was able to (accountable variance 0.74; Givens et al. 1989).

3.2.1.2 Assessment of digestibility

A standard research tool for determining the digestibility of forages is to feed them at maintenance to mature sheep and perform a total faecal collection. This method can be criticized because the animal species and feeding level differ from those to which the results are applied to. The digestibilities of the silages used in this thesis were determined in digestibility trials using sheep and the correlations between the digestibility values determined with cattle were high (SE est. = 2.5 in I, 17.2 in III and 14.8 g/kg DM in IV; Figure 6). The single deviatory points, silage IV in III and IV, can be explained by the considerable amount of concentrate in the cow diets that were absent in diets fed to sheep. It seems that using sheep at maintenance level can successfully be used in evaluating differences in forages harvested at different stages of grass growth even when results are applied to dairy cows.

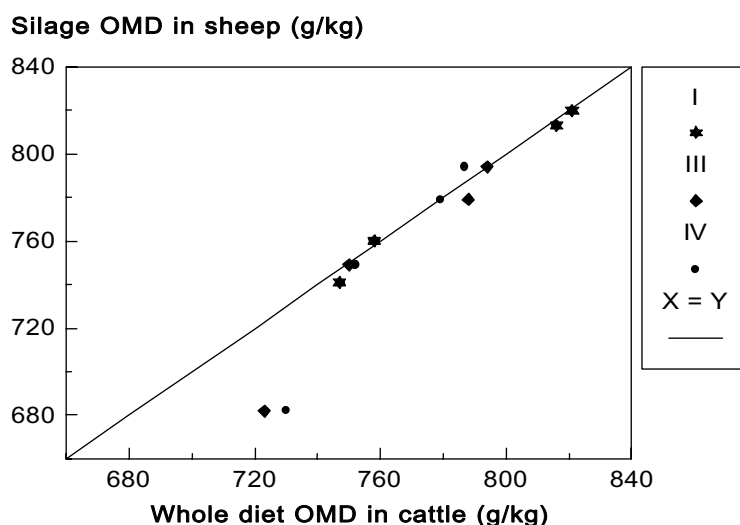


Figure 6. Relationship between OM digestibility in cattle and sheep.

Digestibility trials in sheep can not be conducted routinely even for research purposes, which has led to many different approaches for the estimation of the digestibility of feeds. The digestive processes of a ruminant can be mimicked *in vitro*. Tilley and Terry published their *in vitro* procedure in 1963, which they had developed based on a comparison of several other published methods. It involves an initial anaerobic incubation with rumen fluid for 48 h followed by an incubation with acid pepsin. Recently, a labour saving means to conduct incubation of a batch of feeds sealed in nylon bags in large vessels has been introduced (Ankom Technology Corporation 1998, Holden 1999), although Wilman and Adesogan (2000) noted that precision was compromised compared to the conventional individual tube method.

The problems associated with rumen fluid based methods are that they require the maintenance of rumen fistulated animals as a source of rumen fluid and variations in the quality of rumen fluid are difficult to prevent (Holden 1999). The use of commercially available isolated cellulolytic enzymes overcomes these problems, and several papers reporting the succesful use of such an approach have been published (e.g. Donefer et al. 1963, Jones and Hayward 1975, Pulli 1976,

Friedel 1990, Atwal and Erfle 1993). Mannerkorpi et al. (1992) compared different *in vitro* methods for the prediction of the digestibility of a range of Finnish feed samples. Based on that evaluation, a cellulase based digestion (Friedel 1990) is routinely used in the MTT Animal Nutrition laboratory.

Although one single value representing the correct digestibility of a particular feed does not exist, the values produced with digestibility trials in sheep form the best available basis against which the usefulness of other methods can be evaluated. In the present material (Table 3, Figure 7), the method of Tilley and Terry (1963) considerably underestimated the digestibility compared to reference *in vivo* values. In silage set 1, the difference increased with decreasing maturity, but not in silage set 2. The cellulase method (Friedel 1990) tested only for silage set 2, produced values which were closer to *in vivo* measurements than those derived using Tilley and Terry (1963) method.

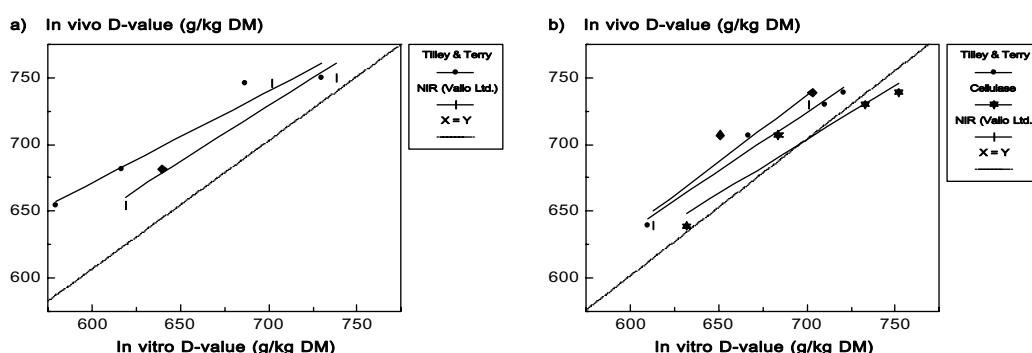


Figure 7. Comparison of *in vitro*/NIRS methods for the prediction of *in vivo* digestibility of silage sets 1 (a) and 2 (b).

For routine feed analysis, even *in vitro* methods are impractical. Feed digestibility values are based either on chemical composition [measured by near infrared reflectance spectroscopy (NIRS) or with wet chemistry techniques] which is connected to feed digestibility utilizing empirical connections, or by estimating digestibility directly by NIRS. In the present material (Figure 7), NIRS estimates of digestibility produced by Valio Ltd. detected differences between feeds well, but it also underestimated the digestibility compared to *in vivo* values. The calibration material at Valio Ltd. has been extended since these measurements were conducted, so that analysis of the same samples using the current prediction equations may yield slightly different values (M. Hellämäki, Valio Ltd., personal communication).

Using only the chemical composition of a sample to estimate its energy value can not be recommended (refer to chapter 3.2.1.1), but NIRS offers great possibilities for routine analysis provided that calibration is properly conducted and that the calibration samples have a sufficiently wide range in forage characteristics under study (Givens et al. 1997, Reeves 2000). NIRS may even be calibrated to directly predict the intake potential of feeds (Park et al. 1997, Gordon et al. 1998, Offer et al. 1998, Steen et al. 1998).

Table 3. Different laboratory methods for the prediction of silage digestibility compared to *in vivo* measurements.

	<i>In vitro</i> rumen fluid ¹⁾			<i>In vitro</i> cellulase ²⁾			NIRS ³⁾		
	R ²	SE est.	Bias ⁴⁾	R ²	SE est.	Bias	R ²	SE est.	Bias
Silage set 1	0.949	41.0	54.3				0.934	24.5	33.0
Silage set 2	0.968	19.9	26.8	0.934	9.7	3.5	0.917	25.4	31.5
Combined	0.874	45.6	40.5				0.912	35.6	32.3

¹⁾According to Tilley & Terry (1963).

²⁾According to Friedel (1990).

³⁾Near infrared reflectance spectroscopy.

⁴⁾Mean overestimation (g/kg DM) compared to reference *in vivo* measurements.

The so called Hohenheim gas test involves the incubation of feed samples in gas tight vessels with rumen fluid, and the amount of gas produced from a sample is used to assess its digestibility (Menke et al. 1979, Menke and Steingass 1988). The gas production method has not proven very useful in determining digestibility directly, but frequent measurements of gas produced have been used to measure the rate of digestion (Pell and Scholefield 1993, Theodorou et al. 1994, Cone et al. 1996, Mauricio et al. 1999). Such estimates were also produced for feed samples of V using a manual apparatus. An alternative approach in measuring the rate of digestibility is to incubate feeds sealed in nylon bags in the rumen for different times and to analyze the loss of feed in relation to time as was done in II and III. The rate of digestion gives some information of the relative differences between feeds and it can be utilized further to calculate ruminal digestibility (Huhtanen et al. 2000a). This requires correct measurements of the required parameters, and use of appropriate mathematical models (Huhtanen and Kukkonen 1995, II), but deserves further attention since improved but relatively simple methods for the characterization of feedstuffs would be extremely valuable.

3.2.2 Protein value

The concentration of CP in forages has traditionally been considered an important indicator of forage quality (chapter 1). This originates probably from the parallel decline of CP concentration and digestibility in the growing crop (Figure 4a). However, CP content cannot be used as a predictor of forage digestibility as discussed in chapter 3.2.1.1.

An other reason contributing to the traditional concept of CP as an indicator of forage quality lies in the history of protein evaluation. Digestible crude protein as an official protein evaluation system in Finland was replaced in 1995 by a Nordic metabolizable protein system, the AAT-PBV system (Madsen et al. 1995) with some specific modifications (Tuori et al. 1998, 2000). The DCP content of a feed is directly dependent on CP concentration. Expressing protein value in terms of amino acids (AA) absorbed from the small intestine (AAT), that consist of both microbial AA synthesized in the rumen and feed AA that escapes rumen degradation, is theoretically more sound.

The discrepancy between CP or DCP concentration in feed and its true protein value is demonstrated by the results of Shingfield et al. (1999). The same ley was fertilized with either 50 or 100 kg N in late spring. The grass was harvested on the same day and although higher N fertilization resulted in higher silage CP content (120 and 148 g/kg DM), effects on D-value were negligible (683 and 671 g/kg DM). Even more importantly, no milk production responses to increases in silage protein were found [29.7 and 29.2 kg energy corrected milk (ECM) production for cows consuming the low and high CP silage, respectively] while responses to rapeseed cake

supplementation were independent of silage CP content.

There was considerably less variation in AAT compared to DCP content of silage in sets 1 and 2. When AAT was expressed per MJ ME in feed DM, the relationship remained stable irrespective of silage harvest date unlike DCP/ME (Figure 8). Calculated AAT values although based on extensive research are still estimates of the true protein value of a feedstuff. However, physiological experiments support the concept that the relationship between energy and protein yielding nutrients to the metabolism of the ruminant animal remains stable when silage digestibility is varied by the timing of harvest (Figure 9a).

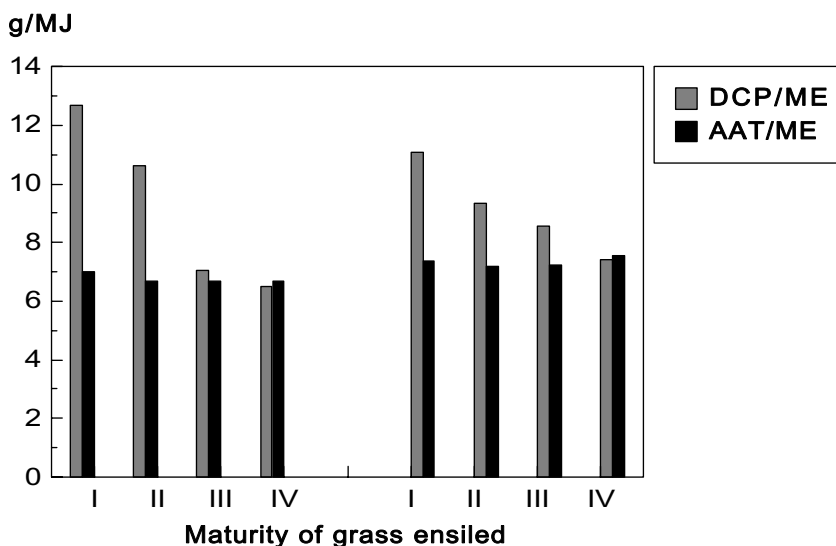


Figure 8. Digestible crude protein (DCP) and amino acids absorbed from the small intestine (AAT) contents of silage in relation to metabolizable energy (ME) concentration, when silages were harvested at different stages of grass growth.

AAT intake was a better predictor of milk protein output than DCP ($R^2 = 0.84$ for DCP and 0.94 for AAT in IV, $n = 4$). The effective protein degradability determined by *in sacco* nylon bag incubations decreased with postponed harvest of grass with values of 0.92, 0.91, 0.87 and 0.85 for silages I to IV, respectively. However, the same effective protein degradability value (0.85) was used for all silages to calculate AAT value according to the official feed evaluation system (Tuori et al. 2000). On the other hand, the digestibility of rumen undegraded feed protein decreases with increasing maturity of grass (Vanhatalo et al. 1996; higher ADF bound N in I; intestinal digestibility of protein determined by the mobile nylon bag method 0.60, 0.53, 0.46 and 0.36 for silages I to IV, respectively in IV). As both these opposing factors are inadequately described, it has been considered more reliable to use constant values for both parameters when calculating AAT values within the official protein evaluation system (Tuori et al. 2000).

The new metabolizable protein systems realize, that AA supply to the animal is greatly influenced by the amount of microbial protein synthesized in the rumen. Microbial protein synthesis in the rumen is primarily related to the amount of fermentable carbohydrates available to microbes (Tuori et al. 2000). Two approaches can be taken to increase the production of microbial AA in the rumen; improve the efficiency of MPS, or increase the amount of fermentable carbohydrates in the rumen by e.g. improved digestibility or increased intake.

The efficiency of MPS is often stated to be lower for silages compared to fresh grass or hay or when silage is fed as the sole feed compared to concentrate or protein supplements (Rooke et al. 1985, van Vuuren et al. 1995). According to the ARC (1984), only 23 g microbial N is synthesized per kg OM apparently digested in the rumen when silage is fed compared to 32 g N when hay or grass is fed. However, Jaakkola and Huhtanen (1993) observed that the efficiency of MPS was greater when restrictively fermented silage was fed compared to barn dried hay. At least three reasons are generally considered to explain the relative inefficiency of silage based diets. Silage fermentation products yield little or no energy to rumen microbes, modifications during ensilage decrease the value of nitrogenous compounds, and energy and N yielding substrates are provided asynchronously to rumen microbes (van Vuuren et al. 1995). It seems that the lower protein value of silage compared to hay or grass should be confined to extensively and/or badly fermented silage (Huhtanen 1998).

MPS was studied in I and IV but with contrasting methods. In I, MPS was calculated based on the flow of purine bases entering the duodenum (Zinn and Owens 1986). In IV, microbial protein was calculated based on urinary purine derivative excretion (Chen and Gomes 1992) in sheep. In both experiments, the intake of silage was restricted on a DM basis. The differences in the level of values was great between experiments (Figure 9a,b) and it may be caused by methodological differences as well as true differences between experiments. It appears that urinary purine derivative excretion can be used to assess relative differences between diets consistent with the ranking based on standard *in vivo* procedures (Shingfield 2000).

Timing of forage harvest did not affect significantly the efficiency of MPS (g microbial N per kg digestible OM intake) in I and IV although values tended to be higher when high digestibility forages were fed (Figure 9a). Figure 9b reveals that if MN was expressed per kg DMI, increasing digestibility increased ruminal MN output more clearly. In practical feeding situations, forage is often fed to ruminants *ad libitum*. As increasing the digestibility of feed also increases voluntary FI (chapter 3.4.1), the microbial N supply to the animal is further increased. In a way, high digestibility of a feed "automatically" means a higher microbial protein supply to the animal, although effects on the efficiency of MPS may not be found.

The concentration of N available to rumen microbes may restrict MPS, i.e. the protein balance in the rumen (PBV) may be excessively low (Madsen et al. 1995). The significance of PBV in cattle nutrition seems to be small (Madsen and Hvelplund 1988) with a few extreme exceptions. According to Huhtanen (1998), calculated PBV values ranging from -15 to -20 g per kg DM have not negatively affected MPS. The N requirement of the microbes also depends on the digestibility of the feed. If the efficiency of MPS is assumed to be 30 g per kg OM apparently digested in the rumen, then a dietary CP concentration of approximately 105 g/kg DM would be sufficient for a D-value of 600 g/kg DM, but a CP concentration of 125 g/kg DM can be efficiently utilized for a D-value of 700 g/kg DM. Results from MTT based on the omasal sampling technique suggest that the true deficit of rumen degradable N is not observed when dietary CP concentrations exceed approximately 125 g/kg DM (Ahvenjärvi et al. 1999).

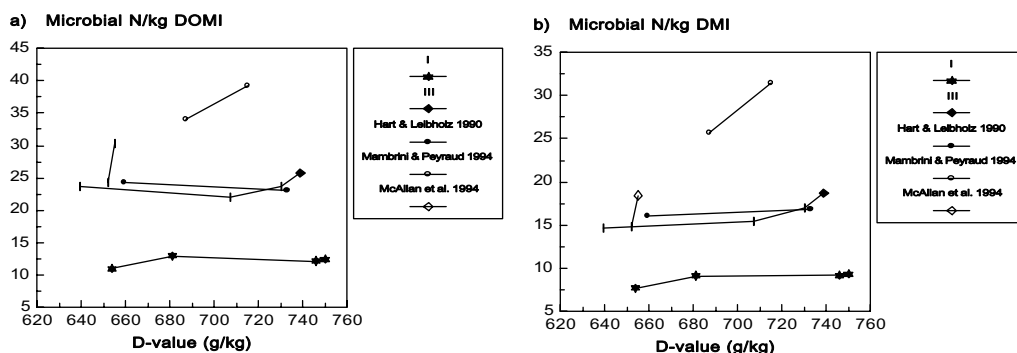


Figure 9. Efficiency of microbial protein synthesis per kg digestible OM intake (a) and per kg DM intake (b).

It is probably more precise to present the N requirement of rumen microbes as ammonia concentrations in rumen fluid. According to Satter and Slyter (1974), optimal MPS would require 3.6 mmol ammonia per litre, while according to Hoover (1986), optimal growth of microbes requires 1.5 mmol per litre and that for optimal fibre degradation 4.7 mmol per litre. Mean rumen ammonia concentrations were clearly higher in I and III even with the latest cut silages, which contained 109 and 113 g CP per kg DM, respectively (Figure 10b). The corresponding diet CP concentrations were 126 and 133. A marked diurnal variation in rumen ammonia concentration is typical for silage-based diets (see Figure 1b in I), and within the feeding cycles when the latest cut silages were fed, the lowest ammonia concentration in I and III was 2.3 and 1.6 mmol per litre, respectively.

Increased N fertilization increases herbage PBV value, while the concentration of AAT remains unchanged (Shingfield et al. 1999). In practice, silage N concentrations are often unnecessarily high leading to reduced efficiency of N use when early cut forages are fed. Dewhurst et al. (1996) reported maximal utilization of silage N in dairy cows when silage had high ME but low CP concentrations. Unfortunately production of such feeds is difficult because of the concomitant decline of both with progressing grass growth. Decreasing the level of N fertilization could be used in this sense (refer to Figure 5), but it may lead to substantial losses in DM yield per hectare.

The optimum concentration of N for maximal growth of grass declines with increasing shoot biomass. Based on work done by Lemaire and Salette (1984), Bélanger and Richards (1997) presented that at even such high DM yields as 5000 kg/ha in the primary growth of grass, optimal CP concentrations based on plant requirements approach 160 g/kg DM. This can be considered adequate in terms of rumen N requirements based on the previous discussion. It seems that if the N supply is adequate to ensure proper growth of grass, no additional concerns should be raised with respect to rumen N availability. Furthermore, protein supplements are generally used in dairy cow rations, so that situations of N deficiency of rumen microbes are rarely encountered in Western countries.

3.3 Physiological responses of ruminants to forage quality

Using forages harvested at different stages of grass maturity may be a convenient method to obtain contrasting feeds to be used in physiological studies when evaluating e.g. basal mechanisms of digestive processes or experimental techniques as was the case for I, II and III. Simultaneously the results can be interpreted for practical use, since variation in forage quality is a major factor affecting the performance of cattle in commercial enterprises.

There are, however, certain problems, which must be taken into account. Forage development is not always straight forward as discussed in chapter 3.1.1, and non-typical development of grass may obscure the results. Also, several characteristics of forages change simultaneously during progressing growth and separating their effects from one another is difficult. Further, animal characteristics may become confounded with experimental treatments, if for example the genetic production potential of animals restricts their responses to increases in nutrient intake.

3.3.1 Rumen fermentation

Although I included restrictively fed young cattle and III dairy cows fed *ad libitum*, the general trends caused by postponed harvest of grass on rumen fermentation were similar (Figure 10). Feeding the more digestible silages decreased rumen pH and increased the concentration of total volatile fatty acids (VFA) in the rumen fluid. The pH was lower and VFA concentration higher in dairy cows reflecting the differences in the feeding level between the experiments. The proportions of different VFA developed similarly with respect to variations in silage D-value in I and III (Figure 10d, e and f). The proportion of acetate decreased while proportion of butyrate increased with increasing silage digestibility, but no discernible effects on propionate were observed.

The responses in other published results have been more variable (Figure 10). Data from Thomas et al. (1980a,b) is based on autumn cut silages with an extended regrowth (42 d) between harvests. Some of the variation in the results of Bosch et al. (1992b) may be explained by the heterogeneity of experimental feeds, i.e. mixing primary growth and regrowth in the same experiment. The grass development was not typical in the data set of McAllan et al. (1994), because in spite of a 26-day difference in harvesting date, there were no differences in *in vivo* diet digestibility when fed to dairy cows. Still the main trends in the combined data are in accordance with the present results.

Rumen fermentation pattern may have important effects on the proportion of different metabolic precursors available to the ruminant animal. The glucogenic to lipogenic VFA ratio was not affected in I or III. The high proportion of butyrate with high digestibility silages could increase the concentration of milk fat, but such a trend was not observed (IV) using the same silages fed in III.

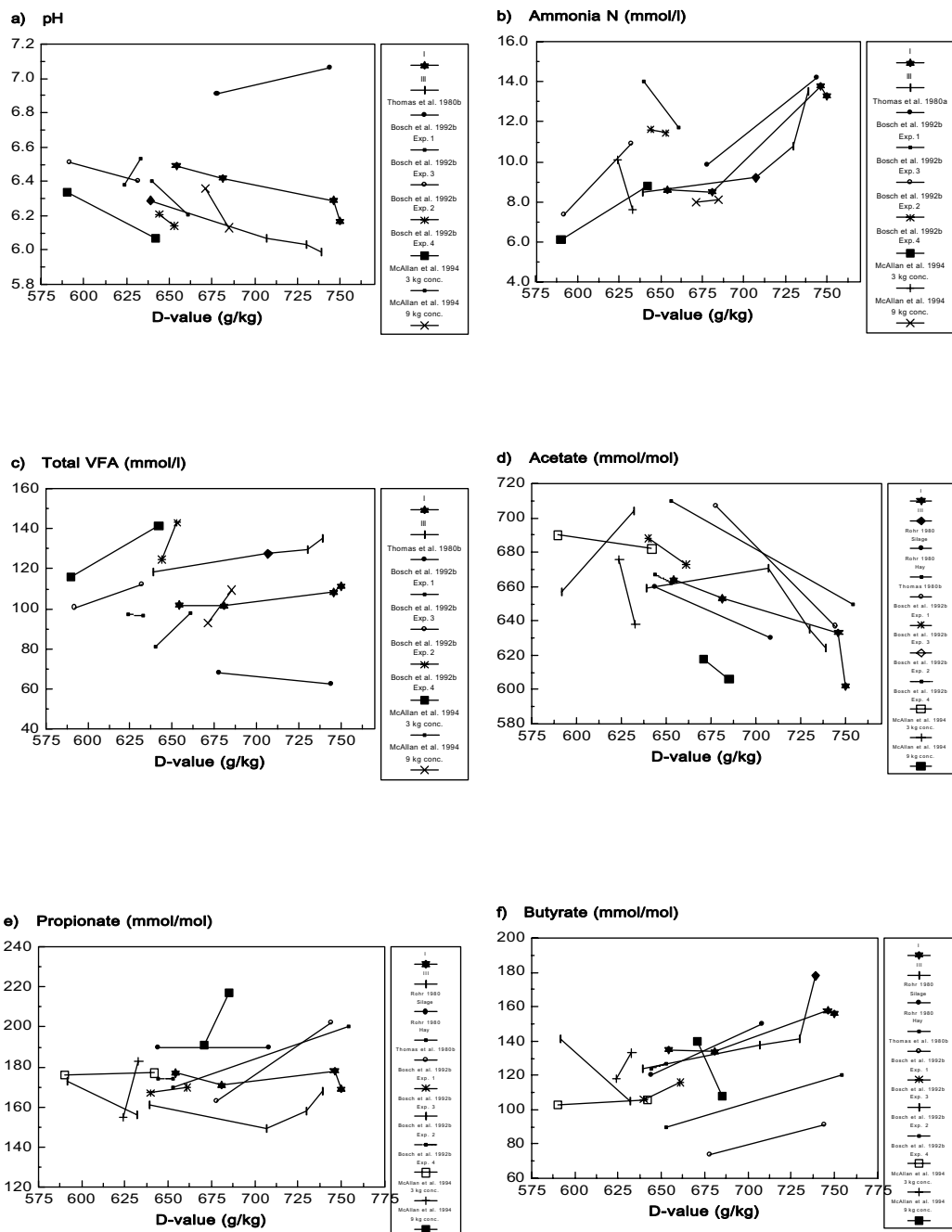


Figure 10. Rumen pH (a), concentrations of ammonia N (b) and VFA (c), and molar proportions of acetate (d), propionate (e) and butyrate (f) in relation to silage D-value.

Cone et al. (1999) concluded, that immature grass is degraded by other microbial populations than mature grass. They fed cows with immature and mature grass and used inoculum from both for the digestion of feeds *in vitro*. When inoculum obtained from cows consuming immature grass was used, digestion of immature grass was faster than that of mature grass, and *vice versa*. The observation that the number of protozoa in rumen fluid decreased with increasing maturity of grass both in I and III supports the concept of rumen flora adaptation that is dependent on forage quality. These differences may be reflected in the proportions of rumen VFA, particularly as a higher proportion of butyrate in total VFA. Williams et al. (2000) physically separated ryegrass leaves into cell walls and cell contents and found a clear decrease in the proportions of propionate and butyrate produced from fermentation of cell contents with increases in leaf maturity. Fermentation pattern from cell walls was rather stable. Increasing numbers of protozoa and increases in the molar proportion of butyrate in rumen VFA were also found when the proportion of concentrate in the diet has been increased (Jaakkola and Huhtanen 1993).

3.3.2 Rumen fill

Increased rumen fill is generally observed with decreases in the digestibility of offered feeds (Gasa et al. 1991, Bosch et al. 1992a, II, III). There also seems to be certain differences in the accumulation of digesta components in the rumen. The largest proportional increases have consistently been observed for indigestible NDF (INDF; Aitchison et al. 1986, Gasa et al. 1991, II, III).

Rumen fill expressed as a proportion of rumen contents for diets based on the lowest digestibility forage is presented in Figure 11. A clear decline in rumen contents with increasing forage digestibility was observed in II and III. The more variable results of Bosch et al. (1992a) may be due to feeds being harvested from different sources of herbage. Results from Gasa et al. (1991) showed similar decreases in rumen DM, NDF and INDF contents in response to forage maturity as II and III but this data is unable to be included in Figure 11 due to the omission of forage digestibility.

In II and III, relative rumen DM fill increased on average 0.009 units per a 10 g/kg decline in D-value. Relative increases in rumen NDF fill was 0.022 units and in INDF fill 0.043 units per 10 g/kg decline in D-value. The trends between II (restricted feeding) and III (*ad libitum* feeding) were almost identical. The results from II with *ad libitum* intake showed less variation in DM and NDF fill, but the results should be interpreted cautiously, since the *ad libitum* feeding period lasted for only 3 days. Results for INDF are not available.

3.3.3 Digestion kinetics

Rumen digestion is a dynamic process, which involves the rates of feed residue digestion (k_d) in the rumen and passage from the rumen (k_p). Feed particles are simultaneously subject to both. Rumen digestibility can be calculated as:

$$\text{Rumen digestibility} = k_d / (k_d + k_p) \quad (\text{Waldo et al. 1972}).$$

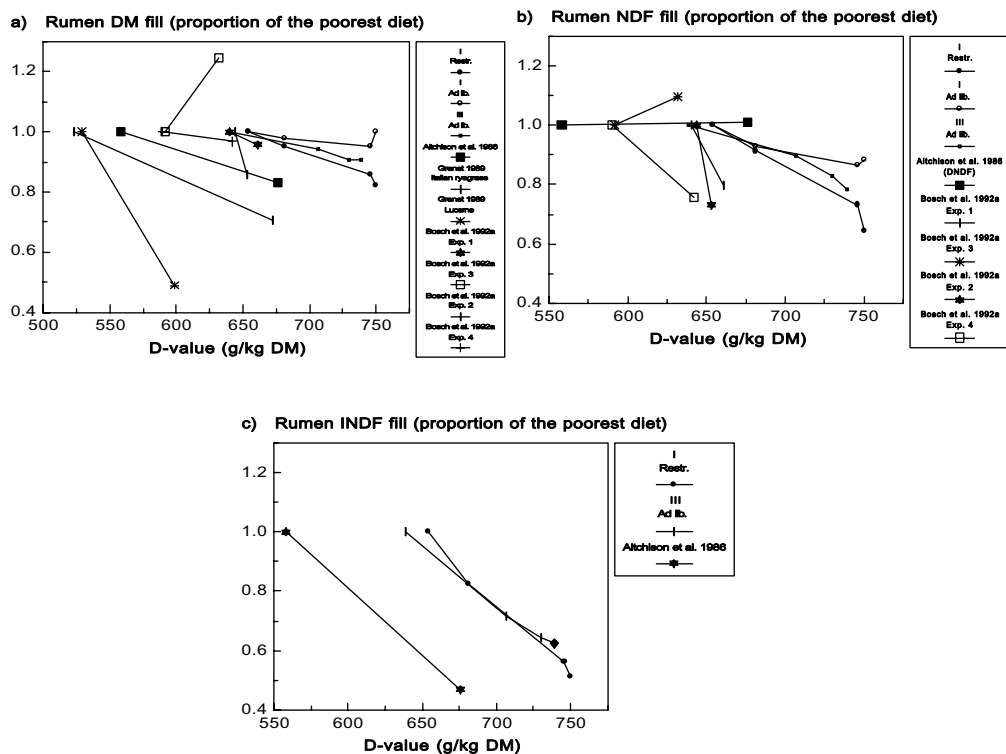


Figure 11. Rumen DM (a), NDF (b) and INDF (c) contents expressed as proportion of the lowest digestibility diet within each experiment in relation to silage D-value.

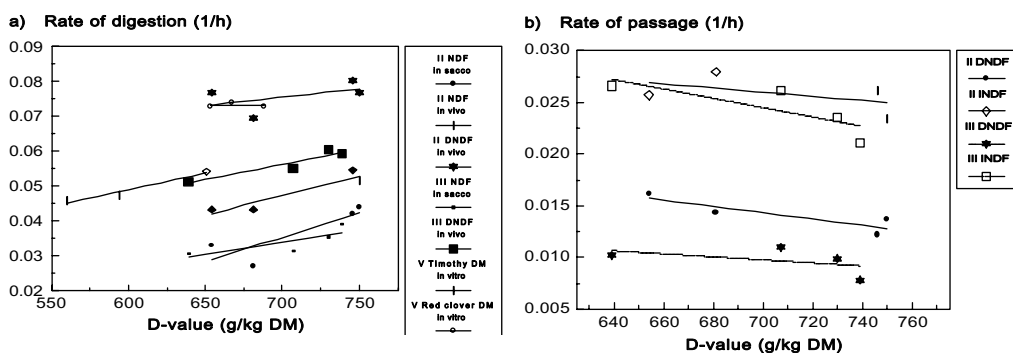


Figure 12. Rates of feed digestion (a) and passage (b) in relation to silage D-value.

The decrease of k_d with increasing maturity of grass was clear (Figure 12a). This has consistently been demonstrated in other studies (Bosch et al. 1992a, Huhtanen and Jaakkola 1994, Cone et al. 1999). On the other hand, k_p increased with increasing grass maturity (Figure 12b, Bosch et al. 1992a, Bosch and Bruining 1995). This probably reflects the greater need to remove indigestible feed residues from the rumen, but still rumen fill increased as discussed in chapter 3.3.2. The extent to which k_p controls FI and rumen fill, or *vice versa* is still obscure (Poppi et al. 2000).

The feed particles in the rumen can be divided into different subclasses. Allen and Mertens (1988) presented a model with two separate particle pools, the non-escapable pool (NEP), which consists of particles not able to leave the rumen by passage, and the escapable pool (EP), which consists of particles leaving the rumen at a certain rate. Particles in NEP must be digested and/or sufficiently comminuted to allow a gradual transfer to the EP.

In II, the retention time of particles in NEP and EP was studied using ytterbium as a marker and interpreting the results with a two-pool model with a gamma time dependency in the first pool. The retention time of particles in NEP increased with increasing maturity of grass, but simultaneously the retention time in EP decreased resulting in no net changes in the total ruminal residence time of particles. Feed particles from high digestibility silage became exposed to passage earlier than those from low digestibility silage, but their rate of passage was slower. This will contribute to a reduction in the digestibility of potentially digestible material in the rumen for early cut silages.

In III, factors in forages that vary with harvesting date that could contribute to different retention times in NEP and EP were studied by separating digesta by wet sieving into large ($>2500\ \mu\text{m}$), medium ($2500\text{--}315\ \mu\text{m}$) and small ($315\text{--}80\ \mu\text{m}$) particles. Particle size is not an adequate measure of its escapability, which is clearly demonstrated by the observation that in general the majority of rumen DM is in the form of particles smaller than that required for ruminal escape (Poppi et al. 1980). However, particle size degradation may contribute significantly to the rate of digestion by exposing digestible components to the activity of microbial enzymes. Progression of digestion then in turn increases the susceptibility of the feed particle to escape the rumen as the functional specific gravity of the particle gradually increases (Mertens 1993). Particle size has also been found to be correlated with k_p (Poppi et al. 1980, McLeod and Minson 1988, Huhtanen et al. 1993a,b, III), which supports its use as a crude estimate of the "escapability" of a particle.

Based on the results in III, factors that are most likely to limit rumen clearance were the reduction of medium to small particles and/or the passage of medium particles from the rumen as they showed the greatest response to postponed harvest. The slower rate of large particle degradation with early cut grass (III) suggests that transfer of particles from the NEP to the EP is independent of harvest date, since the transfer from NEP to EP was slower for late cut grass in II.

According to Ellis et al. (2000), the residence time of particles in NEP remains constant, but that in EP increases when INDF intake increases. This is contrary to the results of II. Unfortunately Ellis et al. (2000) did not define the experimental data sufficiently well so that the means used to manipulate INDF intake and the level of intake constraint remain unclear.

3.3.4 Feed intake

Voluntary FI is the factor, which has the greatest effect on the digestible energy intake of ruminants. According to Mertens (1994), proportionately only 0.1-0.4 of the variation in digestible energy intake can be explained by differences in diet digestibility, while between 0.6 and 0.9 can be attributed to differences in FI. Several different signals have been proposed to control FI of ruminants. They include physical (rumen fill), metabolic [rumen pH, plasma glucose, other metabolites such as rumen acetate and portal propionate, body temperature, body (fat) reserves, oxygen consumption, balance of nutrients] and behavioural factors.

Whether rumen fill and/or k_p control FI or *vice versa* is difficult to assess. For dairy cows fed diets based on high quality silage supplemented with concentrates, silage intake is increased in response to improvements in silage digestibility (chapter 3.4.1). In similar situations, rumen contents have however decreased (Gasa et al. 1991, Bosch et al. 1992a, III) indicating that rumen capacity is not fully exploited when high digestibility forages are fed. The intake potential of the most digestible silage in III would have been 16.9 kg DM/d rather than the 12.5 kg DM/d if rumen fill was the only limitation on intake and digestibility kinetics parameters were unchanged. Hence only the intake of the lowest quality silage can be considered to have been restricted by rumen fill, but whether this was the case, is uncertain. In an earlier study with the same cows, the maximum rumen NDF pool size was greater (8.59 kg) than in III (7.36 kg), which suggests that rumen fill was not tentatively maximal even when late cut silage was fed (III). This interpretation is however subject to error because of differences between diets and stage of lactation.

The "rumen NDF fill restricted intake" of silage I (based on data presented in III) was calculated as follows:

$$\begin{aligned} \text{Estimated potential NDF intake (kg/d)} &= \\ \text{rumen NDF pool (silage IV)} \times k_i \text{ of NDF (silage I)} \times 24 &= 9.98 \text{ kg} \end{aligned}$$

$$\begin{aligned} \text{Additional silage DMI potential (kg/d)} &= \\ (\text{Estimated potential NDF intake} - \text{measured NDF intake}) / \text{NDF concentration in silage I} &= \\ 4.47 \text{ kg} \end{aligned}$$

$$\text{Rumen NDF fill restricted silage I intake} = \text{observed silage I intake} + 4.47 = 16.9 \text{ kg}$$

Mature grass requires long rumen retention times so that microbes have enough time to digest the slowly degradable material. On the other hand, large quantities of feed would have to be consumed to fulfill the energy and nutrient requirements of the animal. This is the major conflict limiting the use of low digestibility forages (Van Soest 1994). Conrad (1964) and later Mertens (1994) have described the intake of gradually improving diets as a bi-phasic, discontinuous equation, where intake is first limited by rumen fill. As forage digestibility gradually improves, a certain point is reached where metabolic factors become limiting when the ability of animals to consume the absorbed energy is attained.

Recently, integrated models of the regulation of FI in ruminants have become more popular. Several different factors are likely to control FI simultaneously (Forbes 1995, Weston 1996, Ellis et al. 1999). In their latest review, Ellis et al. (2000) emphasized the balance of nutrients, especially the supply of essential AA to ruminant tissues, as the major intake regulating factor. This is somewhat contrary to the previous concepts emphasizing the role of energy in the control FI (e.g. Mertens 1994, Van Soest 1994).

However, this apparent conflict may not be great, because for ruminants the intake of energy and protein are unable to be separated as individual entities. Early cut grass has a high energy content associated with a low rumen fill, but it also provides more AA via MPS to the host animal, so that it is difficult to identify the stimulus driving intake. As depicted in figures 8 and 9, the relationship between AAT and ME supply remains stable irrespective of harvesting date, i.e. digestibility of the grass. In an unpublished data set from MTT covering 15 silages harvested over 5 years, calculated AAT concentration increased 8.1 g per a 1 MJ increase in ME concentration. The former supports an ECM production of 178 g, the latter 194 g (Tuori et al. 2000) showing that the balance between AA and ME supply for milk production is maintained irrespective of the time of silage harvest. If the CP concentration of the forage is increased by N fertilization, no intake responses have been found, but intestinal AA supply is also unlikely to increase (Vanhatalo and Toivonen 1993, Peyraud et al. 1997). Furthermore, when protein supplementation is used with silage based diets, increases in intake are often observed (Thomas 1987, Chamberlain et al. 1989, Huhtanen 1998, Rinne et al. 1999b, chapter 3.4.3).

Weston (1996) presented a conceptual model linking rumen function and energy metabolism in the regulation of FI. This model seems to permit an integration of most aspects presented in the literature and those observed for the current data. The model is presented in Figure 13. Weston (1996) presented net energy intake as the X-axis, but for the current interpretation purposes this can be considered to equal forage D-value. When forage D-value increases, the role of limitations due to rumen digesta load decreases while those of energy increase [the line of asterisks (*) in Figure 13]. In the model, observed FI is a result from an interplay between ruminal load and energy deficit.

Weston (1996) realized that deficiencies of essential nutrients and/or other palatability constraints may limit FI. If such constraints are removed, both rumen fill and FI are increased. This is illustrated in Figure 13 as the line of circles (o) and represents situations where e.g. protein supplements elicit FI responses (see chapter 3.4.3).

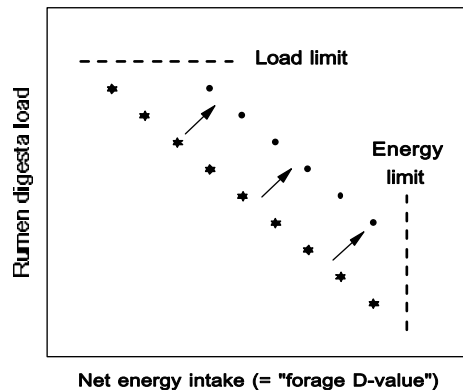


Figure 13. Conceptual model of feed intake according to Weston (1996). The horizontal dashed line represents the limit imposed by rumen digesta load, and the vertical dashed line the limit imposed by animal energy requirements. The asterisks (*) indicate that generally neither of them limits feed intake, but as the net energy intake of animals increases, the importance of energy limit becomes progressively more important. If some constraint related e.g. to balance of nutrients or palatability is removed, both intake and rumen digesta load are increased simultaneously as indicated by the arrows directed towards the circles (o).

3.4 Effects of the timing of forage harvest on milk production

3.4.1 Responses to forage digestibility

The economic significance of forest harvest time is realised when forages varying in digestibility are fed to productive ruminants. Compiling data from different experiments gives the possibility to produce numerical estimates of economically important production responses. Such reviews covering milk production of dairy cows have previously been published (Castle 1975, Thomas 1980, Huhtanen 1994). To extend the data and its processing, a new data set was collected and the results are currently documented.

Data was collected from 17 publications (including IV) reporting milk production and FI when forages harvested at different stages of grass growth were fed *ad libitum* to dairy cows. Data in the form of mean values for each silage is presented in Table 4. Most experiments also included other dietary factors, and in such cases, data within each treatment (sub-experiment) were recorded separately resulting in 125 observations in total. Linear regression analysis was conducted using the MIXED procedure of SAS using experiment as a fixed factor and sub-experiment within study as a random factor. The results are presented in Table 5.

Table 4. Description of published data used for linear regression analysis.

Ref.	Silage		Intake (kg DM/d)		Production (kg/d)		Concentration in milk (g/kg)		
	Maturity	D-value	Silage	Total	Milk	ECM	Protein	Fat	Lactose
(1) Aston, Thomas, Daley, Sutton and Dhanoa 1994									
	1	756	9.6	17.1	22.6	21.9	30.8	39.5	45.9
	2	645	8.4	15.9	21.1	20.5	30.3	40.1	45.9
(2) Bertilsson and Burstedt 1983									
I	1	703	14.1	19.2	23.4	24.8	34.5	44.4	
	2	678	12.8	18.0	21.0	22.7	35.0	46.0	
II	1	691	14.7	20.8	24.3	25.9	35.3	44.5	
	2	589	12.5	18.7	23.1	23.3	32.0	42.0	
(3) Castle, Retter and Watson 1980									
	1	650	9.6	14.8	18.6	18.1	32.0	38.7	47.0
	2	624	9.0	14.2	18.4	18.1	32.0	39.7	46.9
(4) Castle, Gill and Watson 1981									
	1	645	8.9	14.4	19.5	19.5	32.1	40.7	
	2	603	8.8	14.2	18.3	18.8	32.4	43.1	
(5) Castle, Gill and Watson 1983									
	1	694	12.6	15.2	21.2	21.2	31.7	40.7	47.4
	2	633	11.1	13.6	18.9	18.8	30.9	41.0	47.3
(6) Davies and Clench 1987									
	1	725	12.1	16.0	22.1	22.2	32.5	40.9	47.4
	2	665	9.9	13.8	17.8	16.7	31.5	37.4	44.5
(7) Ettala, Rissanen and Lampila 1978 (Exp.1)									
	1	644	10.1	15.6	19.1	20.1	34.7	42.5	49.7
	2	630	9.5	14.4	17.6	19.0	34.5	44.8	49.8
(8) Gordon 1980a									
	1	726	10.7	18.3	26.2	25.3	32.5	37.5	47.8
	2	699	10.0	17.6	25.0	23.6	31.4	36.3	47.6
	3	617	8.1	15.7	23.3	21.8	30.7	36.5	46.9
(9) Gordon 1980b									
	1	707	9.3	18.1	23.6	23.6	34.7	39.9	45.9
	2	624	8.6	17.4	22.0	22.4	34.7	41.1	46.0
(10) Gordon, Porter, Mayne, Unsworth and Kilpatrick 1995									
	1	678	10.2	19.0	24.1	23.2	32.2	38.0	
	2	632	8.6	17.3	21.9	21.6	31.5	40.5	
(11) Heikkilä, Väättäinen and Toivonen 1993									
	1	713	10.7	18.2	24.8	26.9	33.1	46.0	50.1
	2	666	10.5	17.8	23.4	24.9	32.6	44.5	50.1
(12) Kristensen and Skovborg 1990									
Exp. 1	1	756	10.6	17.2	23.3	22.3	31.2	38.3	
	2	667	9.5	16.1	21.7	20.1	30.4	36.7	
	3	637	9.1	15.7	20.1	18.6	29.7	36.5	
Exp. 2	1	717	10.2	16.5	25.9	25.3	30.9	40.1	
	2	680	9.2	15.4	23.2	22.7	30.7	40.3	
	3	604	8.8	15.2	21.3	21.0	31.2	40.3	
Exp. 3	1	756	12.4	18.8	25.4	26.0	33.0	42.8	
	2	631	9.0	15.4	21.2	21.0	31.1	41.1	
	3	601	8.9	15.3	21.1	20.4	31.3	39.0	
Exp. 4	1	685	10.1	16.7	20.5	20.4	30.3	41.9	
	2	619	9.5	16.1	21.0	20.2	30.1	39.4	
	3	613	10.1	16.7	20.8	19.8	29.9	38.9	
(13) Moisey and Leaver 1984 (Exp. 2)									
	1	674	9.7	16.0	20.6	21.1	34.1	41.4	47.7
	2	603	9.4	15.6	17.9	18.7	33.4	43.7	47.1
(14) Phipps, Weller and Bines 1987									
	1	680	6.1	12.4	16.7	15.9	32.8	37.1	46.4
	2	600	5.6	11.9	15.6	14.5	31.1	36.4	46.6
(15) Rinne, Jaakkola, Kaustell, Heikkilä and Huhtanen 1999 (IV)									
	1	739	12.4	19.8	27.3	30.8	33.4	50.4	48.2
	2	730	12.2	19.5	26.5	30.1	34.0	50.5	48.0
	3	707	11.7	19.2	26.0	29.7	33.1	51.2	48.1
	4	639	10.8	18.3	23.5	25.9	32.7	48.6	48.2
(16) Steen and Gordon 1980									
	1	670	9.4	18.5	27.6	26.5	31.8	37.3	48.2
	2	628	8.5	17.5	25.2	24.1	31.2	37.4	48.3
(17) Thomas, Daley, Aston and Hughes 1981									
	1	748	9.9	16.2	28.0	26.5	31.5	36.1	48.5
	2	639	9.5	15.8	24.7	24.4	29.4	41.0	48.2

Table 5. Relationships between silage quality and milk production variables based on linear regression analysis (n=125).

	Intercept	Slope	P-value	Root MSE	R ² (1)	R ² (2)
D-value (10 g/kg DM):						
Intake per day						
Silage DM (kg)	-0.26	0.156	0.0001	0.443	0.954	0.713
DM (kg)	6.21	0.154	0.0001	0.441	0.964	0.695
ME (MJ)	-30.4	3.29	0.0001	5.39	0.970	0.881
AAT (g)	10.8	20.3	0.0001	41.9	0.973	0.849
CP (g)	-419.0	48.3	0.0001	159.7	0.897	0.646
Production per day						
Milk (kg)	3.86	0.270	0.0001	0.810	0.939	0.693
ECM (kg)	0.88	0.317	0.0001	0.824	0.959	0.760
Protein (g)	-32.5	11.0	0.0001	26.4	0.951	0.780
Fat (g)	-21.1	10.2	0.0001	36.5	0.966	0.772
Concentration in milk (g/kg)						
Protein	25.2	0.104	0.0001	0.487	0.910	0.587
Fat	34.5	0.101	0.0035	1.208	0.921	0.326
CP concentration (10 g/kg DM):						
Intake per day						
Silage DM (kg)	7.59	0.169	0.0003	0.706	0.883	0.272
DM (kg)	13.9	0.172	0.0002	0.694	0.911	0.243
ME (MJ)	132.8	3.72	0.0001	12.5	0.837	0.358
AAT (g)	1054	33.6	0.0001	82.7	0.894	0.413
Production per day						
Milk (kg)	16.3	0.366	0.0001	1.16	0.873	0.368
ECM (kg)	16.3	0.380	0.0001	1.34	0.892	0.364
Protein (g)	485	14.3	0.0001	43.8	0.866	0.395
Fat (g)	685	14.6	0.0003	61.6	0.902	0.340
Concentration in milk (g/kg)						
Protein	30.5	0.105	0.0075	0.611	0.858	0.350
Fat	42.5	-0.078	0.3221	1.253	0.915	0.274

(1) Correlation coefficient describing model fit (experiment included as a variable).

(2) Correlation coefficient describing the proportion of within experiment variation accounted for by D-value and CP concentration, respectively.

Model prediction errors were higher when silage CP concentration rather than D-value was used as the predictor, which is in accordance with the earlier discussion. Because the majority of the variation in the data set is caused by variation between different experiments, correlation coefficients are very high [R² (1) in Table 5]. The correlation coefficient describing variation within experiment [R² (2) in Table 5] clearly shows that silage D-value is better than silage CP concentration for the prediction of the production potential of a feed. Other silage qualities like fibre concentration could not be evaluated due to a lack of comprehensive information in the original publications.

From a practical point of view, the goal of forage evaluation is to accurately describe the production potential of feeds. In IV, ECM production was most accurately predicted from silage *in vivo* D-value measured with sheep (R²=0.99). *In vitro* based D-value measurements were also good predictors of milk output [R²=0.92 when measured according to Tilley and Terry (1963) and

$R^2=0.88$ with the cellulase method (Friedel 1990)]. However, more traditional chemical entities of CP and crude fibre were less accurate ($R^2=0.78$ and 0.60), while NDF was even poorer ($R^2=0.54$). It seems that attempts to measure digestibility directly rather than to estimate feeding value through chemical analyses are worthwhile (refer to chapter 3.2.1.1.).

The numerical responses in the current data were, in general, similar to those reported in the earlier reviews. In the present data set, milk production increased 0.27 kg/d per 10 g/kg increase in D-value, that is consistent with values of 0.23 kg/d (Castle 1975), 0.29 kg/d (Thomas 1980) and 0.26 kg/d (Huhtanen 1994). The similarity of the values is partly based on the fact that some same data is used in different calculations.

The general trends between silage D-value and production responses seem clear, but the magnitude of response is subject to considerable variation. Although in general the results from IV are in good accordance with the figures presented in Table 5, milk production responses were higher than the mean value derived in the collected data set, i.e. 0.50 kg ECM per a 10 g/kg increase in D-value. The smallest response in the data set was obtained in the experiment of Castle et al. (1980) in which the increase in ECM production was only 0.02 kg, while Davies and Clench (1987) obtained the highest response of 0.92 kg ECM per 10 g/kg increase in D-value.

Variable silage fermentation characteristics may introduce variation in the response by modifying silage intake potential (van Vuuren et al. 1995, Huhtanen et al. 2000b). Although general effects of grass maturity on silage fermentation have not been detected (chapter 3.1.3), in certain cases, important differences in the fermentation quality affecting silage intake may be found. In this particular case, the early cut silage of Davies and Clench (1987) could be judged to be of better fermentation quality compared to late cut silage (DM 270 vs. 216 g/kg, pH 3.8 vs. 4.3 and proportion of ammonia in total N 58 vs. 88 g/kg for early and late cut silages, respectively). The only silage fermentation quality parameter reported by Castle et al. (1980) was silage pH (3.99 for early and 4.26 for late cut silage, respectively). This does not allow definite conclusions to be made concerning silage fermentation quality particularly as the silages also differed in DM concentration (251 and 319 g/kg for early and late cut silages, respectively).

An other factor which will affect the magnitude of response to forage D-value is the proportion of concentrate in the diet. Obviously the quality of forage will have a greater effect the higher the proportionate amount in the diet. The proportion of concentrate was approximately 0.27 in the experiment of Davies and Clench (1987) and 0.36 in the experiment of Castle et al. (1980). Possible other factors causing variability in production responses include limitations in the genetic potential of cows consuming higher quality diets, deficiencies in other essential nutrients, or inaccuracies in the characterization of D-value.

Increased energy and nutrient intake caused partly by increased energy and nutrient concentrations in silage and partly by increased intake of silage explain the production responses to improved silage digestibility. Silage intake increased by 0.156 kg, ME intake by 3.29 MJ and AAT intake by 20.3 g per day per 10 g/kg DM increase in D-value.

In the present data set, SDMI increased linearly with increasing silage digestibility across the entire range of D-values. The level of concentrate supplementation used in most experiments was moderate (on average 0.371), which would push the limit of metabolic control further. According to the data set of Rook et al. (1991), SDMI was quadratically influenced by silage D-value. Their simple quadratic regression model gave the maximum SDMI at a D-value of 750 g/kg DM. However, the significance of the quadratic trend is relatively small, since silages with D-value above 750 g/kg DM are rarely produced in practice.

A great deal of research has been directed towards the modelling the silage intake of cattle (e.g. Rook et al. 1991 including evaluation of several earlier models). Many of the models have included silage characteristics, but the importance of silage digestibility has been variable. In the data set of Rook et al. (1991) with silages of good fermentation quality, silage D-value was among the most important variables affecting SDMI. Recently, large studies examining the intake potential of silages have been conducted in sheep and dairy cows (Offer et al. 1998) and in beef cattle (Steen et al. 1998). Silage digestibility was identified as an important factor in controlling FI, although a higher accuracy of prediction could be produced by directly estimating intake potential with NIRS in both studies.

Increasing D-value increased the concentrations of protein and fat in the milk although milk fat responses were more variable. Lactose concentration was not evaluated due to missing values. Protein concentration was increased in 67 % and fat concentration in 53 % of the comparisons. Thomas (1984) and Huhtanen (1994) also reported an increase in protein concentration with increases in silage digestibility, but the effects on fat concentration were subject to variation. Higher protein concentration of milk associated with feeding high digestibility silage increases the value of milk as a raw material for subsequent processing.

3.4.2 Interactions between silage D-value and concentrate supplementation

Silage is rarely used as the sole feed because of its suboptimal intake potential and the inability to provide sufficient energy and AA to meet the requirements of intensive milk production. As a result, concentrate supplementation of silages of variable harvesting dates has been actively studied. In 10 experiments (numbers 1, 2, 3, 5, 6, 12, 13, 14, 15, 16 in Table 4) of the current data set, different levels of concentrate supplementation were used. These experiments were used to examine the interactions between silage D-value and concentrate supplementation. The results are presented in Table 6.

In the current data set, the interaction between silage D-value and concentrate supplementation was negative, but the value was small and insignificant. Figure 15a illustrates that the greater the digestibility of silage, the higher the reduction in SDMI following concentrate supplementation. Substitution rates (decrease in SDMI per a 1 kg increase in concentrate DMI) for silages with D-values of 600, 650, 700 and 750 were 0.40, 0.42, 0.45 and 0.47, respectively. The relatively high mean substitution rate indicates that concentrates do not truly supplement silage based diets, but they replace silage in the diet. Substitution rates currently obtained correspond well with previously published values of 0.52 (Thomas 1987), 0.41 (Rook et al. 1991) and 0.39 (Ryhänen et al. 1996).

Table 6. Association between silage D-value (10 g/kg DM), concentrate supplementation (kg DM) and their interaction on production variables based on a linear regression analysis (n=94).

	Intercept	D-value	P-value	Conc.	P-value	Interact.	P-value	Root MSE	R ²
Intake per day									
Silage	0.318	0.189	0.0001	-0.133	0.7018	-0.00447	0.3891	0.552	0.941
DM (kg)	2.247	0.160	0.0001	-0.433	0.0001			0.556	0.940
DM (kg)	0.307	0.189	0.0001	0.868	0.0148	-0.00449	0.3884	0.555	0.948
	2.245	0.160	0.0001	0.567	0.0001			0.557	0.948
ME (MJ)	-133.8	4.09	0.0001	15.36	0.0004	-0.1112	0.0754	6.62	0.958
	-85.8	3.37	0.0001	7.90	0.0001			6.76	0.957
Production per day									
Milk (kg)	-11.3	0.432	0.0001	2.46	0.0001	-0.0266	0.0035	0.95	0.916
	0.19	0.260	0.0001	0.675	0.0001			1.00	0.907
ECM (kg)	-15.5	0.500	0.0001	2.57	0.0001	-0.02905	0.0023	0.99	0.938
	-3.00	0.312	0.0001	0.627	0.0001			1.05	0.930
Protein (g)	-587.2	16.8	0.0001	89.0	0.0001	-0.9397	0.0027	32.5	0.928
	-181.4	10.7	0.0001	26.0	0.0001			34.4	0.919
Fat (g)	-723.0	22.1	0.0001	110.3	0.0004	-0.1319	0.0036	47.1	0.936
	-154.0	13.6	0.0001	21.9	0.0001			49.8	0.929
Concentration in milk (g/kg)									
Protein	21.0	0.145	0.0005	5.84	0.1291	-0.0056	0.3254	0.607	0.861
	23.5	0.109	0.0001	2.08	0.0001			0.611	0.859
Fat	32.0	0.157	0.1055	0.111	0.9039	-0.0056	0.6828	1.466	0.861
	34.5	0.121	0.0021	-0.264	0.0009			1.468	0.861

Although substitution rate can not be directly attributed to silage digestibility, the slight increase in the substitution rate with increasing quality of silage seems reasonable in the context of earlier reports (Thomas 1987, Forbes 1995, Ryhänen et al. 1996). According to Thomas (1987), the intake of silage as a sole feed is a better index of substitution rate than silage digestibility. The intake of silage as a sole feed is however difficult to estimate, and at least in the case of equivalent silages in terms of fermentation quality, digestibility is highly correlated with silage intake potential. Use of the silage intake index, which combines silage digestibility with fermentation quality for the prediction of silage intake potential (Huhtanen et al. 2000b) deserves further attention as a means to estimate substitution rate.

In production parameters, interactions between D-value and concentrate supplementation were significant (Figure 14b and c). Responses of ECM and milk protein output to concentrate supplementation diminished the higher the silage D-value. However, numerical differences were small. The milk production response per kg concentrate DMI was 0.68 kg when silage D-value was 650 g/kg compared to 0.54 kg when silage D-value was 700 g/kg. The difference of only 0.15 kg indicates that the ability to compensate for poor silage digestibility with concentrate supplements is limited. Corresponding responses derived for the comparison of silages would be 1.6 kg ECM.

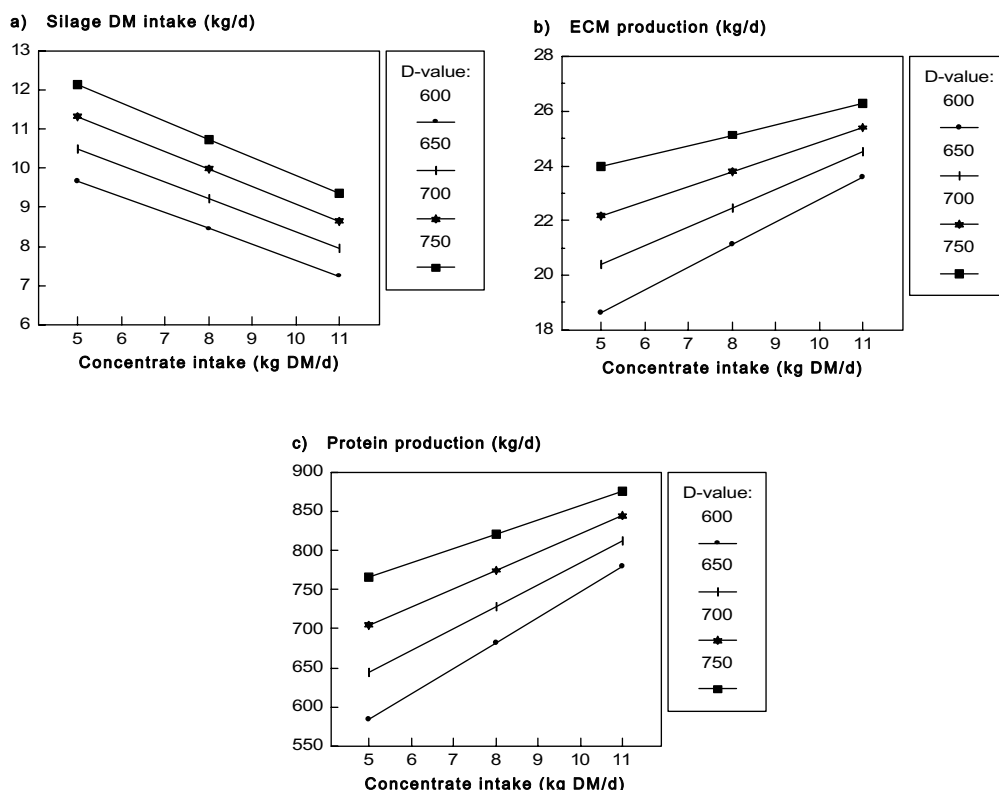


Figure 14. Schematic representation of the interactions presented in Table 6 between silage D-value and concentrate supplementation for silage DM intake (a), energy corrected milk production (b) and milk protein output (c).

The average ECM response per 1 kg additional concentrate intake in the current data set was 0.63 kg. Most experiments in the current data set were conducted with relatively low levels of concentrate supplementation (mean proportion of concentrate DMI of total DMI was 0.371). This is reasonable in order to elucidate differences between forages, and also to achieve situations where the intake of high quality forages is not so readily restricted by the genetic production potential of the cows. The milk production response to increased concentrate supplementation has been found to decrease at higher levels of supplementation (Huhtanen 1998).

Since a 10 g/kg DM increase elicited an ECM production response of 0.27 kg, an increase of 20 g/kg in D-value would be required to compensate for a 1 kg decrease in concentrate DMI. Based on the average daily decline in herbage D-value of 5 g/kg DM, this would mean a difference of 4 days in the harvest time of grass to compensate for the use of 1 kg concentrate in the ration. In IV, 1 kg of additional concentrate DM increased ECM production by 0.55 kg while an increase in D-value was essentially equal (0.50 kg). Hence a difference of only 2.3 days in harvest time was equivalent to the production potential of 1 kg of additional concentrate DM.

3.4.3 Interactions between silage D-value and protein supplementation

This chapter focuses on improved protein supply to dairy cows via increased use of dietary protein supplements. Studies where the CP intake of cows has been increased due to increased N fertilization of grass are excluded because no production responses have been found in such experiments (Huokuna 1968, Ettala et al. 1971, 1974, Keady et al. 1995, Shingfield et al. 1999).

Interactions between silage harvest time and protein supplementation were not statistically evaluated, because only a few studies examining this phenomena could be found. The data reported by Castle et al. (1983) was included in the data set, although the amount (1.5, 3 or 4.5 kg/d) of a high protein (363 g CP/kg DM) supplement was studied rather than CP content. The lack of data is somewhat surprising because early cut grass has been considered an important source of protein to cattle albeit without a critical evaluation of this approach.

In this data set, milk production increased on average by 0.84 kg, milk protein output by 31.4 g and silage intake by 0.20 kg per 10 g/kg DM increase in diet CP concentration. Responses were independent of silage D-value (Figure 15). Results reported by Thomas et al. (1984) confirm that protein supplementation is worthwhile even with silages of high D-value and CP concentration. Actually, in that experiment, clear responses to protein supplementation were only achieved with the highest D-value silage. Unfortunately the data can not be included into Figure 15 because of the inadequate description of experimental silages. In the study of Heikkilä et al. (1993), higher level of protein supplementation had a negative effect on milk production. This is somewhat unexpected, because responses to protein supplementation have generally been positive and relatively consistent (Huhtanen 1998).

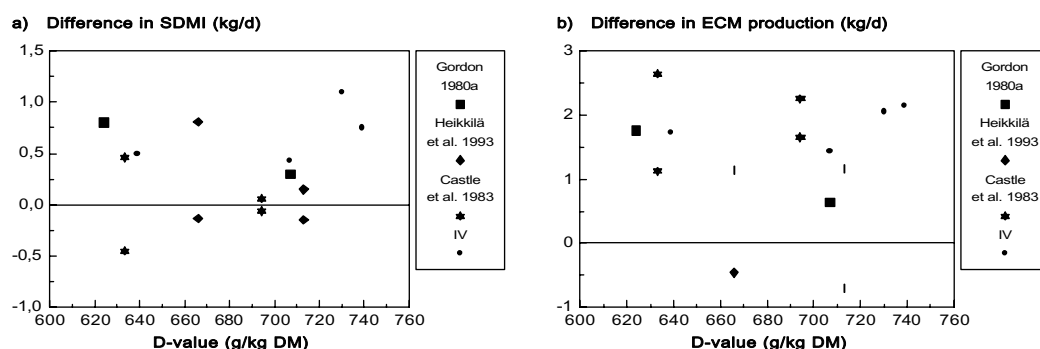


Figure 15. Responses in silage DM intake (a) and energy corrected milk production (b) to protein supplementation in relation to silage D-value.

As milk production responses to protein supplementation are likely even when high D-value (and high CP content) silages are used, use of ample protein supplementation may be profitable in such cases. The high production responses are connected to smaller substitution rates of high protein concentrates, which justifies their use especially in situations when a high silage intake is targeted (Thomas 1987, Chamberlain et al. 1989).

Several hypotheses have been presented to explain FI and milk production responses to protein supplementation. They include increased AA supply to the host tissues either due to increased supplies of rumen undegradable protein or as a result of a higher efficiency of MPS. Ellis et al. (2000) emphasized the necessity of an adequate CP supply to the rumen, because according to them, MPS is first limited by some molecular precursors associated with ruminal hydrolysis of dietary CP. If that is true, it seems that grass CP is not a very good source of these compounds, while hydrolysis of protein supplements is. Protein supplements may elicit positive effects on rumen environment such as decreased acid load in the rumen fluid (R. Dewhurst, IGER, Aberystwyth, UK; personal communication). According to Ahvenjärvi et al. (1999), rapeseed feed supplementation did not increase MPS in the rumen, but undegraded feed protein flow to the intestines was increased that was associated with higher milk production. It is possible that restrictively fermented silages generally fed in Finland differ from more extensively fermented silages in respect to MPS in the rumen.

An other approach relies on differences in diet digestibility and/or rate of digestion, which would alleviate the rumen fill constraints. According to a review of 13 comparisons, rapeseed feed supplementation did not affect diet digestibility (Huhtanen 1998), so that metabolic effects either due to increased supply or a better balance of absorbed AA seem a more likely explanation.

The only experiment in which silage harvest time was examined concurrently with both concentrate and protein supplementation was IV. The design of this experiment allows comparisons of the efficiency of ME and AAT utilization to be conducted, when their intake was manipulated by different dietary means (Table 7). The results indicated that additional ME was used most efficiently for milk production when energy intake was increased with protein supplements. This supports the earlier discussions of the positive metabolic effects of increased intestinal AA supply. The ME derived from improved silage digestibility was used more efficiently than that derived from increases in concentrate supplementation. Probable explanations include the negative effects of increased concentrate supplementation on ruminal digestion and partition of nutrients towards tissues rather than towards milk (Huhtanen 1998).

The differences between the ME efficiency values calculated based on the official feed evaluation system (Tuori et al. 2000; silage D-value determined in sheep) and those determined *in vivo* in cows show that the present feed evaluation system lacks the dynamic properties necessary to account for associative effects between feeds, which are required for an accurate description of nutrient supply. The additional ME intake was overestimated both for earlier harvested silage and increases in concentrate allowance, the extent of which was greater for the latter. The situation was reversed when ME intake was increased by protein supplementation. The theoretical value based on the official feed value system is 0.195 (Tuori et al. 2000).

The efficiency of conversion of additional AAT intake into milk protein was highest, when the intake of AAT was increased by earlier harvesting of grass. The efficiency increased further, if the unnecessarily early harvested silage I was excluded. Increasing the level of concentrate or protein concentration in concentrate supplements were equal in this respect.

Table 7. Comparison of the efficiency of ME utilization (kg ECM produced per MJ additional ME) when ME intake was manipulated by different dietary means, and when the ME intake was calculated based on either feed table values (ME_{TAB}) or from the intake of digestible organic matter determined *in vivo* (ME_{DET}). Adopted from IV.

Dietary means to increase ME or AAT intake	Eff. of ME utilization		ME _{TAB} / ME _{DET}	Eff. of AAT utilization
	ME _{TAB}	ME _{DET}		
Earlier harvest of silage	0.138	0.151	0.914	0.59
Earlier harvest of silage ¹⁾	0.146	0.159	0.917	0.78
Increased concentrate allowance	0.104	0.137	0.759	0.50
Higher CP conc. of concentrate	0.244	0.190	1.284	0.49

¹⁾Excluding silage I.

4 CONCLUSIONS AND SUGGESTED FUTURE RESEARCH

Conclusions

- * The daily decline in D-value of the gramineous plants was on average 4.9 g/kg DM in the present material demonstrating that the timing of harvest has a great effect on forage quality. In two data sets, the decline in D-value was distinctly curvi-linear with respect to time further emphasizing the importance of harvesting date.
- * The rate of decline in D-value in red clover was only half of that in grasses showing that leguminous and gramineous forage species should be considered independently.
- * Correct estimation of forage digestibility in particular is an essential component of planning sensible feeding regimens. This is necessary both for economic reasons and environmental aspects in order to reduce nutrient load entering the environment due to inefficient nutrient utilization. Chemical components of grass (i.e. crude protein and fibre concentrations) inadequately predicted production potential, emphasizing the importance of reliable estimation of forage digestibility for feed evaluation.
- * The nutrient supply to the ruminant is modified by the pregastric microbial fermentation so that the provision of nutrients to the animal can not be assessed solely on the basis of feed composition. Digestive processes of ruminants were clearly affected by changes in forage quality caused by differences in the timing of primary growth harvest. Forage digestibility associated with modifications of rumen fermentation affected the supply of nutrients to the ruminant more clearly than changes in forage CP content, which was reflected by variations in urinary N excretion.

- * Postponed harvest of grass resulted in decreased feed, energy and nutrient intake and reduced milk production of dairy cows. Based on a literature review described in the present thesis, silage intake was decreased by 0.156 kg, energy corrected milk production by 0.32 kg and protein concentration in milk by 0.104 g/kg in response to a decline of 10 g/kg DM in silage D-value.
- * Although the interaction between silage D-value and concentrate supplementation was significant for production parameters for literature data, the magnitude of responses was numerically minor. Thus possibilities to compensate for poor silage digestibility by increases in concentrate feeding are limited. Increases in concentrate CP content elicited high production responses irrespective of silage D-value.
- * The efficiency of utilization of marginal increases in ME intake were greater, when achieved by earlier harvest of grass compared to increased concentrate allowance. The highest efficiency was obtained, when ME intake was manipulated by protein supplementation suggesting that responses were mediated via metabolic advantages either from increased AAT supply or from an improved balance of absorbed AA.

Suggested future research

There are certain areas which would benefit from further research so that harvesting strategies of forages and therefore forage based feeding systems can be optimized.

- * The general pattern of the development of grass primary growth is well known, but its quantitative control at the farm level is generally difficult. An advisory service to assist in the timing of primary growth of grass inspired by this work has been developed (Artturi 2000, Rinne et al. 2000b), and this process should be continued. This requires an integration of animal and crop sciences.
- * Forage resources should be optimized over the whole growing season, i.e. more work concerning grass regrowth is urgently required. Both aspects of forage production and *in vivo* production potential of forages need to be addressed. Biological data from different harvesting systems would allow an economical assessment of various strategies.
- * Development of dynamic feed evaluation systems are necessary in order to account for associative effects between feeds. This will allow supplementation of forages varying in quality and available in different quantities in an economical and/or biologically efficient manner.

References

- Ahvenjärvi, S., Vanhatalo, A., Huhtanen, P. & Varvikko, T. 1999. Effects of supplementation of a grass silage and barley diet with urea, rapeseed meal and heat-moisture-treated rapeseed cake on omasal digesta flow and milk production in lactating dairy cows. *Acta Agric. Scand, Sect. A, Animal Sci.* 49: 179-189.
- Aitchison, E.M., Gill, M., Dhanoa, M.S. & Osbourn, D.F. 1986. The effect of digestibility and forage species on the removal of digesta from the rumen and the voluntary intake of hay by sheep. *Br. J. Nutr.* 56: 463-476.
- Allen, M. S. and Mertens, D. R. 1988. Evaluating constraints on fiber digestion by rumen microbes. *J. Nutr.* 118: 261-270.
- Ankom Technology Corporation. 1998. Daisy^{II} Incubator. Cited 26 September 2000. Updated 1 March 1998. Available from Internet: <http://www.ankom.com/products/daisy.html>.
- ARC. 1984. Report on the protein group of the Agricultural Research Council working party on the nutrient requirements of ruminants. Commonwealth Agricultural Bureaux, Farnham, UK.
- Artturi. 2000. D-value estimates based on a grass growth model are distributed to Finnish farmers through this web site. Cited 26 September 2000. Available from Internet: <http://www.agronet.fi/artturi/english.html>.
- Aston, K., Thomas, C, Daley, S.R., Sutton, J.D. & Dhanoa, M.S. 1994. Milk production from grass silage diets: effects of silage characteristics and the amount of supplementary concentrate. *Anim. Prod.* 59: 31-41.
- Atwal, A.S. & Erfle, J.D. 1993. Effect of progressive maturity of timothy on digestibility by fungal cellulases. *J. Agric. Food Chem.* 41: 2309-2314.
- Beever, D.E., Offer, N. & Gill, M. 2000. The feeding value of grass and grass products. In: *Grass - Its production and utilization*. Ed. A. Hopkins. Blackwell Science Ltd, UK. 3rd ed. pp. 140-195.
- Bélanger, G. & McQueen, R.E. 1996. Digestibility and cell wall concentration of early- and late-maturing timothy (*Phleum pratense* L.) cultivars. *Can. J. Plant Sci.* 76: 107-112.
- & McQueen, R.E. 1998a. Analysis of the nutritive value of timothy grown with varying N nutrition. *Grass Forage Sci.* 53: 109-119.
- & McQueen, R.E. 1998b. Leaf and stem nutritive value of timothy cultivars differing in maturity. *Can. J. Plant Sci.* 78: 237-245.
- & Richards, J.E. 1997. Growth analysis of timothy grown with varying N nutrition. *Can. J. Plant Sci.* 77: 373-380.
- Bertilsson, J. & Burstedt, E. 1983. Effects of conservation method and stage of maturity upon the feeding value of forages to dairy cows. *Swedish J. Agric. Res.* 13: 189-200.
- Bosch, M.W. & Bruining, M. 1995. Passage rate and total clearance rate from the rumen of cows fed on grass silages differing in cell-wall content. *Br. J. Nutr.* 73: 41-49.
- , Lammers-Wienhoven, S. C. W., Bangma, G. A., Boer, H. and van Adrichem, P. W. M. 1992a. Influence of stage of maturity of grass silage on digestion processes in dairy cows. 2. Rumen contents, passage rates, distribution of rumen and faecal particles and mastication activity. *Livest. Prod. Sci.* 32: 265-281.
- , Tamminga, S., Post, G., Leffering, C.P. & Muylaert, J.M. 1992b. Influence of stage of maturity of grass silage on digestion processes in dairy cows. 1. Composition, nylon bag degradation rates, fermentation characteristics, digestibility and intake. *Livest. Prod. Sci.* 32: 245-264.
- Castle, M.E. 1975. Silage and milk production. *Agric. Progr.* 50: 53-60.
- , Gill, M.S. & Watson, J.N. 1981. Silage and milk production: a comparison between grass silages of different chop lengths and digestibilities. *Grass Forage Sci.* 38: 31-37.
- , Gill, M.S. & Watson, J.N. 1983. Silage and milk production: a comparison between three rates of high-protein concentrate supplementation of grass silage of two digestibilities. *Grass Forage Sci.* 38: 135-140.
- , Retter, W.C. & Watson, J.N. 1980. Silage and milk production: a comparison between three grass silages of different digestibilities. *Grass Forage Sci.* 35: 219-225.
- Chamberlain, D.G., Martin, P.A. & Robertson, S. 1989. Optimizing compound feed use in dairy cows with high intakes of silage. In: *Recent advances in animal nutrition* (ed. W. Haresign & D.J.A. Cole). pp. 175-193. Butterworths, London.
- Chen, X. B. and Gomes, M. J. 1992. Estimation of microbial protein supply to sheep and cattle

- based on urinary excretion of purine derivatives - an overview of the technical details. Occasional Publication 1992. 20 p. International Feed Resources Unit, Rowett Research Institute, Aberdeen, UK.
- Cherney, D.J.R., Cherney, J.H. & Lucey, R.F.** 1993. In vitro digestion kinetics and quality of perennial grasses as influenced by forage maturity. *J. Dairy Sci.* 76: 790-797.
- Cone, J.W., Gelder, A.H. van, Soliman, I.A., Visser, H. de & Vuuren, A.M. van.** 1999. Different techniques to study rumen fermentation characteristics of maturing grass and grass silage. *J. Dairy Sci.* 82: 957-966.
- , Gelder, A.H. van, Visscher, G.J.W & Oudshoorn, J.** 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. *Anim. Feed Sci. Technol.* 61: 113-128.
- Conrad, H.R., Pratt, A.D. & Hibbs, J.W.** 1964. Regulation of feed intake in dairy cows. I. Change in importance of physical and physiological factors with increasing digestibility. *J. Dairy Sci.* 47: 54-62.
- Davies, I.** 1976. Developmental characteristics of grass varieties in relation to their herbage production. 1. An analysis of high-digestibility varieties of *Dactylis glomerata* at three stages of development. *J. Agric. Sci., Camb.* 87: 25-32.
- Davies, O.D. & Clench, S.F.** 1987. Effects of silage quality and level of concentrate on the performance and dry matter intakes of autumn calving dairy cows. 8th Silage Conference, Hurley, UK. pp. 171-172.
- Deinum, B., de Beijer, J., Nordfeldt, P.H., Kornher, A. Østgard, O. & van Bogaert, G.** 1981. Quality of herbage at different latitudes. *Neth. J. Agric. Sci.* 29: 141-150.
- Dewhurst, R.J., Mitton, A.M., Offer, N.W. & Thomas, C.** 1996. Effects of the composition of grass silages on milk production and nitrogen utilization by dairy cows. *Anim. Sci.* 62: 25-34.
- Donefer, E., Nieman, P.J., Crampton, E.W. & Lloyd, L.E.** 1963. Dry matter disappearance by enzyme and aqueous solutions to predict the nutritive value of forages. *J. Dairy Sci.* 46: 965-970.
- Ellis, W.C., Poppi, D. & Matis, J.H.** 2000. Feed intake in ruminants: Kinetic aspects. In: *Farm animal metabolism and nutrition*. CAB International. pp. 335-363.
- , Poppi, D.P., Matis, J.H., Lippke, H., Hill, T.M. & Rouquette, F.M.** 1999. Dietary-digestive-metabolic interactions determining the nutritive potential of ruminant diets. In: *Proc. Vth International Symposium on the Nutrition of Herbivores*. American Society of Animal Science, IL, USA. pp. 423-481.
- Ettala, E., Poutiainen, E., Lampila, M., Rinne, K. & Takala, M.** 1971. Tyypilannoituksen vaikutus laidunnurmeen ja lehmien tuotoksiin (in Finnish). *Kehittyvä Maatalous* 4: 18-29.
- , Rissanen, H. & Lampila, M.** 1978. Säilörehun korjuuasteen vaikutus lehmien tuotantoon (in Finnish). *Kehittyvä maatalous* 38: 17-33.
- , Takala, M. & Lampila, M.** 1974. Tyypilannoitustasot lypsylehmien säilörehuruokinnassa (in Finnish). *Kehittyvä Maatalous* 18: 51-59.
- Fagerberg, B. & Ekbohm, G.** 1995. Variation in clover content and in nutritional value of grass-clover leys. In: *Crop Production Science 23*. Department of Crop Production Science, Swedish University of Agricultural Sciences, Uppsala, Sweden. 46 p.
- Fick, G.W., Wilkens, P.W. & Cherney, J.H.** 1994. Modeling forage quality changes in the growing crop. In: *Forage quality, evaluation, and utilization* (ed. G.C. Fahey). ASA, CSSA & SSSA, Madison, WI, USA.
- Forbes, J. M.** 1995. Voluntary food intake and diet selection in farm animals. CAB International, Wallingford, Oxon, UK. 532 p.
- Frame, J.** 1987. The effect of strategic fertilizer nitrogen and date of primary harvest on the productivity of a perennial ryegrass/white clover sward. *Grass Forage Sci.* 42:33-42.
- Friedel, K.** 1990. Die Schätzung des energetischen Futterwertes von Grobfutter mit Hilfe einer Cellulase-methode. *Wissenschaftliche Zeitung Universität Rostock, N-Reihe* 39: 78-86.
- Gasa, J., Holtenius, K., Sutton, J.D., Dhanoa, M.S. & Napper, D.J.** 1991. Rumen fill and digesta kinetics in lactating Friesian cows given two levels of concentrates with two types of grass silage *ad lib.* *Br. J. Nutr.* 66: 381-398.
- Givens, D.I., De Boever, J.L. & Deaville, E.R.** 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. *Nutr. Res. Rev.* 10: 83-114.
- , Everington, J.M. & Adamson, A.H.** 1989. The digestibility and metabolisable energy content of grass silage and their prediction from laboratory measurements. *Anim. Feed Sci. Technol.* 24: 27-43.
- Gordon, F.J.** 1980a. The effect of silage type on the performance of lactating cows and the response to high

- levels of protein in the supplement. *Anim. Prod.* 30: 29-37.
- , 1980b. The effect of interval between harvests and wilting on silage for milk production. *Anim. Prod.* 31: 35-41.
- , **Cooper, K.M., Park, R.S. & Steen, R.W.J.** 1998. The prediction of intake potential and organic matter digestibility of grass silages by near infrared spectroscopy analysis of undried samples. *Anim. Feed Sci. Technol.* 70: 339-351.
- , **Porter, M.G., Mayne, C.S., Unsworth, E.F. & Kilpatrick, D.J.** 1995. Effect of forage digestibility and type of concentrate on nutrient utilization by lactating dairy cattle. *J. Dairy Res.* 62: 15-27.
- Grenet, E.** 1989. A comparison of the digestion and reduction in particle size of lucerne hay (*Medicago sativa*) and Italian ryegrass hay (*Lolium italicum*) in the ovine digestive tract. *Br. J. Nutr.* 62: 493-507.
- Hakkola, H. & Nykänen-Kurki, P.** 1994. Effect of nitrogen fertilization and cutting time on the quality and variable cost of red clover and timothy herbage production. In: Mannetje, L. 't & Frame, J. (Ed.). *Grassland and Society. Proceedings of the 15th General Meeting of European Grassland Federation*, Wageningen, The Netherlands, June 6-9, 1994. p. 105-108.
- Hart, F.J. & Leibholz, J.** 1990. The effect of species of grass, stage of maturity and level of intake on the degradation of protein and organic matter in the rumen of steers. *Aust. J. Agric. Res.* 41: 791-798.
- Heikkilä, T., Toivonen, V. & Huhtanen, P.** 1998. Effect of spring and autumn silage, protein and concentrate level on milk production. *Proc. 17th General meeting of EGF*, Debrecen, Hungary. pp. 717-721.
- , **Väättäinen, H. & Toivonen, V.** 1993. Effects of acid and biological additives on grass silage quality and milk production in dairy cows supplemented with concentrates containing three levels of rapeseed meal. *Proc. 10th International Silage Conference*, Dublin, Ireland. pp. 190-191.
- Holden, L.A.** 1999. Comparison of methods of in vitro dry matter digestibility for ten feeds. *J. Dairy Sci.* 82: 1791-1794.
- Homb, T.** 1953. Chemical composition and digestibility of grassland crops. *Acta Agric. Scand.* 3: 1-32.
- Hoover, W.H.** 1986. Chemical factors involved in ruminal fiber digestion. *J. Dairy Sci.* 69: 2755-2766.
- Huhtanen, P.** 1994. Forage influences on milk composition. *Proceedings of Nova Scotia Forage Conference; Forage: Seeding to feeding. The Nova Scotia Forage Council*, Dartmouth, N.S., Canada. pp. 144-162.
- , 1998. Supply of nutrients and productive responses in dairy cows given diets based on restrictively fermented silage. *Agric. Food Sci. Finl.* 7: 219-250.
- , **Ahvenjärvi, S. & Heikkilä, T.** 2000a. Effects of sodium sulphate and potassium chloride fertilizers on the nutritive value of timothy grown on different soils. *Agric. Food Sci. Finl.* 9: 105-119.
- , **Dakowski, P. & Vanhatalo, A.** 1993a. Composition, digestibility and particle-associated enzyme activities in rumen digesta as influenced by particle size and time after feeding. *J. Anim. Feed Sciences* 1: 223-235.
- & **Jaakkola, S.** 1994. Influence of grass maturity and diet on ruminal dry matter and neutral detergent fibre digestion kinetics. *Arch. Anim. Nutr.* 47: 153-167.
- , **Khalili, H., Nousiainen, J.I., Jaakkola, S., Heikkilä, T. & Nousiainen, J.** 2000b. Prediction of intake potential of unwilted grass silage by dairy cows. *Proc. Br. Soc. Anim. Sci.* 2000. Scarborough, UK.
- & **Kukkonen, U.** 1995. Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. *Anim. Feed Sci. Technol.* 52: 141-158.
- , **Kukkonen, U., Jaakkola, S. & Kallio, M.** 1993b. The effect of particle size on passage rate of digestible and indigestible neutral detergent fiber. *J. Dairy Sci.* 76 (Suppl. 1): 282 (Abstr.).
- Huokuna, E.** 1968. Lypsykarjan laitumen runsas typpilannoitus [in Finnish, summary: Heavy nitrogen fertilization of milk cattle pasture]. *Ann. Agric. Fenn.* 7: 25-32.
- & **Hakkola, H.** 1984. Koiranheinän ja timotein kasvu ja rehuarvon muutokset säilörehuasteella. *Tiedote* 8/84. Maatalouden tutkimuskeskus, Jokioinen. 54 p.
- Jaakkola, S. & Huhtanen, P.** 1993. The effects of forage preservation method and proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. *Grass Forage Sci.* 48: 146-154.
- , **Toivonen, V. & Huhtanen, P.** 1999. Effects of nitrogen fertilization of grass on fermentation in untreated and formic acid treated silage. *Proc. XIIth International Silage Conference*. July 5-7, 1999. Uppsala, Sweden. pp. 164-165.
- Jones, D.I.H. & Hayward, M.V.** 1975. The effect of pepsin treatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. *J. Sci. Food Agric.* 26: 711-718.
- Keady, T.W.J., Mayne, C.S. & Fitzpatrick, D.A.** 2000. Prediction of silage feeding value from the

- analysis of the herbage at ensiling and effects of nitrogen fertilizer, date of harvest and additive treatment on grass silage composition. *J. Agric. Sci., Camb.* 134: 353-368.
- , **Murphy, J.J. & Fitzgerald, S.** 1995. The effects of level of nitrogen fertilizer application to silage swards on subsequent silage composition, intake and animal performance of lactating dairy cattle. *Anim. Sci.* 60: 515 (Abstr.).
- Khalili, H., Huhtanen, P., Jaakkola, S., Varvikko, T.** 1995. The effects of ruminal and duodenal casein infusion on dry matter (DM) intake of red clover silage and rumen pool size, digestion and passage kinetics of neutral detergent fibre (NDF). *Ann. Zootech.* 44 (Suppl.): 243 (Abstr.).
- Kivimäe, A.** 1959. Chemical composition and digestibility of some grassland crops. *Acta Agric. Scand. Suppl.* 5. 142 p.
- Kristensen, V.F. & Skovborg, E.B.** 1990. Effect of cutting time on yield and quality of grass and on silage intake and milk production in dairy cows. *Fællesberetning nr. 15. Statens Planteavl- og husdyrbrugsforsøg, Tjele, Denmark.* 37 p.
- Lampila, M. & Ettala, E.** 1971. Tillfredsställande av mjölkkons proteinbehov huvudsakligen med protein från vallfoder (in Swedish). Fortryck av föredrag, Seksjon V husdyrbruk. NJF Congress 29 June - 2 July 1971. pp. 53-68.
- Lemaire, G. & Salette, J.** 1984. Relation entre dynamique de croissance et dynamique de prélèvement d'azote pour un peuplement de graminées fourragères. 1. Etude de l'effet du milieu. *Agronomie* 4: 432-430. Ref. Bélanger & Richards (1997).
- Lindgren, E. & Lindberg, J.E.** 1988. Influence of cutting time and N fertilization on the nutritive value of timothy. 1. Crude protein content, metabolizable energy and energy value determined *in vivo* vs. *in vitro*. *Swedish J. Agric. Res.* 18: 77-83.
- Maaseutukeskusten liitto.** 2000. Tarkkailulehmien rehunkulutus 1972-1999. Cited 6 November 2000. Available from Internet: <http://www.maaseutukeskus.fi/tuotantotuloksia/rehunkulutus72-99.htm>.
- Madsen, J. & Hvelpund, T.** 1988. The influence of different protein supply and feeding level on pH, ammonia concentration and microbial protein synthesis in the rumen of cows. *Acta Agric. Scand.* 38: 115-125.
- , **Hvelpund, T., Weisbjerg, M.R., Bertilsson, J., Olsson, I., Spörndly, R., Harstad, O. M., Volden, H., Tuori, M., Varvikko, T., Huhtanen, P. and Olafsson, B .L.** 1995. The AAT/PBV protein evaluation system for ruminants. A revision. *Norwegian J. Agric. Sci., Suppl.* 19. 37 p.
- Mambrini, M. & Peyraud, J.L.** 1994. Mean retention time in digestive tract and digestion of fresh perennial ryegrass by lactating dairy cows: influence of grass maturity and comparison with maize silage. *Reprod. Nutr. Dev.* 34: 9-23.
- Mannerkorpi, P., Suvitie, M., Tuori, M. & Hellämäki, M.** 1992. A comparison of different laboratory techniques for the prediction of the nutritive value of forages. *Proc. 14th General Meeting of EGF, Lahti, Finland.* pp. 599-601.
- Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S. & Theodorou, M.K.** 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. *Anim. Feed Sci. Technol.* 79: 321-330.
- McAllan, A.B., Sutton, J.D., Beever, D.E. & Napper, D.J.** 1994. Rumen fermentation characteristics and duodenal nutrient flow in lactating cows receiving two types of grass silage with two levels of concentrates. *Anim. Feed Sci. Technol.* 46: 277-291.
- McDonald, P., Henderson, A.R. & Heron, S.J.E.** 1991. *The Biochemistry of Silage*. 2nd ed. Chalcombe Publications, Bucks, UK. 340 p.
- McLeod, M.N. & Minson, D.J.** 1988. Large particle breakdown by cattle eating ryegrass and alfalfa. *J. Anim. Sci.* 66: 992-999.
- Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W.** 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gasproduction when they are incubated with rumen liquor *in vitro*. *J. Agric. Sci., Camb.* 93: 217-222.
- & **Steingass, H.** 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* 28: 7-55.
- Mertens, D. R.** 1993. Kinetics of cell wall digestion and passage in ruminants. In *Forage cell wall structure and digestibility* (ed. H. G. Jung, D. R. Burton, R. D. Hatfield and J. Ralph). ASA-CSSA-SSSA, Madison, WI. pp. 535-570.
- . 1994. Regulation of forage intake. In *Forage quality, evaluation, and utilization* (ed. G. C. Fahey, Jr., M. Collins, D. R. Mertens and L. E. Moser). ASA-CSSA-SSSA, Madison, WI. pp. 450-493.

- Moisey, F.R. & Leaver, J.D.** 1984. A study of two cutting strategies for the production of grass silage for dairy cows. *Res. Dev. Agric.* 1: 47-52.
- Nissinen, O. & Hakkola, H.** 1995. Effects of plant species and harvesting system on grassland production in northern Finland. *Agric. Sci. Finl.* 4: 479-494.
- Nykänen, A.** 2000. Apilanurmen korjuuaika [in Finnish, Harvesting time of clover leys] Cited 26 September 2000. Available from Internet: <http://www.agronet.fi/luomu/apila/nettisivu.html>.
- Offer, N.W., Percival, D.S., Dewhurst, R.J. & Thomas, C.** 1998. Prediction of voluntary intake potential of grass silage by sheep and dairy cows from laboratory silage measurements. *Anim. Sci.* 66: 357-367.
- Park, R.S., Gordon, F.J., Agnew, R.E., Barnes, R.J. & Steen, R.W.J.** 1997. The use of Near Infrared Reflectance Spectroscopy on dried samples to predict biological parameters of grass silage. *Anim. Feed Sci. Technol.* 68: 235-246.
- Pell, A.N. & Scholefield, P.** 1993. Computerized monitoring of gas production to measure forage digestion in vitro. *J. Dairy Sci.* 35: 1063-1073.
- Peyraud, J.L., Astigarraga, L. & Faverdin, P.** 1997. Digestion of fresh perennial ryegrass fertilized at two levels of nitrogen by lactating dairy cows. *Anim. Feed Sci. Technol.* 64: 155-171.
- Phipps, R.H., Weller, R.F. & Bines, J.A.** 1987. The influence of forage quality and concentrate level on dry matter intake and milk production of British Friesian heifers. *Grass Forage Sci.* 42: 49-58.
- Poppi, D.P., France, J. & McLennan, S.R.** 2000. Intake, passage and digestibility. In: Feeding systems and feed evaluation models (ed. M.K. Theodorou & L. France). CAB International. pp. 35-52.
- , Norton, B.W., Minson, D.J. & Hendricksen, R.W.** 1980. The validity of the critical size theory for particles leaving the rumen. *J. Agric. Sci., Camb.* 94: 275-280.
- Pulli, S.** 1976. Cellulase digestion technique compared with the *in vitro* digestibility of forages. *J. Sci. Agric. Soc. Finl.* 48: 187-194.
- ,** 1980a. Growth factors and management technique used in relation to the developmental rhythm and yield formation pattern of a clover-grass stand. *J. Sci. Agric. Soc. Finl.* 52: 215-280.
- ,** 1980b. Growth factors and management technique used in relation to the developmental rhythm and yield formation pattern of a pure grass stand. *J. Sci. Agric. Soc. Finl.* 52: 281-330.
- Pärssinen, P.** 1999. Määrää vai laatua - nurmikasviljalostajan näkökulma (Abstract: Discrepancy between yield and quality of timothy). Maatalouden tutkimuskeskuksen julkaisuja Sarja A 67. pp. 71-80.
- Reeves, J.B., III.** 2000. Use of near infrared reflectance spectroscopy. In: Farm Animal Metabolism and Nutrition (ed. J.P.F. D'Mello). CAB International, UK. pp. 185-207.
- Rinne, M., Hellämäki, M., Nousiainen, J., Aura, E. & Huhtanen, P.** 1999a. Development of timothy during progressing growth and subsequent nutritional implications. Proc. XIIth International Silage Conference. July 5-7, 1999. Uppsala, Sweden. p. 166-167.
- , Hellämäki, M., Nousiainen, J., Aura, E. & Huhtanen, P.** 2000a. Kevätkorjuun optimoinnista koko kesän nurmisadon hallintaan (in Finnish). Maataloustieteen päivät 2000, Kotieläintiede. Maaseutukeskusten liiton julkaisuja no 952. p. 126-129.
- , Jaakkola, S., Varvikko, T. & Huhtanen, P.** 1999b. Effects of the type and amount of rapeseed feed on milk production. *Acta Agric. Scand., Sect. A, Animal Sci.* 49: 137-148.
- , Nousiainen, J., Mattila, I., Nikander, H. & Huhtanen, P.** 2000b. D-value estimates based on grass growth model are distributed to Finnish farmers via Internet. Accepted to XIX International Grassland Congress, Brazil, February 2001.
- Rohr, K.** 1980. Digestion and metabolism of forage. In: Forage conservation in the 80's. Ed. C. Thomas. Occasional Symposium No. 11, British Grassland Society. pp. 96-106.
- Rook, A.J., Gill, M., Willink, R.D. & Lister, S.J.** 1991. Prediction of voluntary intake of grass silages by lactating cows offered concentrates at a flat rate. *Anim. Prod.* 52: 407-420.
- Rooke, J.A., Brett, P.A., Overend, M.A. & Armstrong, D.G.** 1985. The energetic efficiency of rumen microbial protein synthesis in cattle given silage-based diets. *Anim. Feed Sci. Technol.* 13: 255-267.
- Ryhänen, M., Huhtanen, P., Jaakkola, S. & Ahvenjärvi, S.** 1996. EU-jäsenyyden vaikutus maidontuotantoon (in Finnish). In: Maatalousyritysten sopeutuminen EU:ssa vallitseviin hintasuhteisiin (ed. M. Yläalo). Univ. of Helsinki, Department of Economics and Management, Publications No. 12, Agricultural economics. pp. 75-118.
- Salo, M.-L.** 1978. Kasvuasteen ja tyyppilannoituksen vaikutus säilörehunurmen rehuarvoon (in Finnish). *Kehittyvä Maatalous* 38: 3-9.
- , Nykänen, A. & Sormunen, R.** 1975. Nurmikasvien koostumus, pepsiini-HCl-liukoisuus ja in vitro-sulavuus eri kasvuasteilla (in Finnish, Abstract: Composition, pepsin-HCl solubility and in vitro

- digestibility of forages at different growth stages). *J. Sci. Agric. Soc. Finl.* 47: 480-490.
- Satter, L.D. & Slyter, L.L.** 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.* 32: 199-208.
- Shingfield, K.J.** 2000. Estimation of microbial protein supply in ruminant animals based on renal and mammary purine metabolite excretion. A review. *J. Anim. Feed Sci.* 9: 169-212.
- , **Jaakkola, S. & Huhtanen, P.** 1999. Comparison of the effects of dietary nitrogen supplements on the intake and milk production of Finnish Ayrshire dairy cows fed grass silage-based diets. *Proceedings of the XIIth International Silage Conference, Uppsala, Sweden, July 5-7, 1999.* p. 159-160.
- Steen, R.W.J. & Gordon, F.J.** 1980. The effect of type of silage and level of concentrate supplementation offered during early lactation on total lactation performance of January/February calving cows. *Anim. Prod.* 30: 341-354.
- , **Gordon, F.J., Dawson, L.E.R., Park, R.S., Mayne, C.S., Agnew, R.E., Kilpatrick, D.J. & Porter, M.G.** 1998. Factors affecting the intake of grass silage by cattle and prediction of silage intake. *Anim. Sci.* 66: 115-127.
- Syrjälä, L. & Ojala, R.** 1978. Kevät- ja syysasadosta eri kehitystasasteilla valmistetun timoteisäilörehun ravintoarvo (in Finnish). *Kehittyvä Maatalous* 39: 36-49.
- Syrjälä-Qvist, L., Pekkarinen, E., Setälä, J. & Kangasmäki, T.** 1984. Effect of nitrogen fertilization on the protein quality of timothy grass and silage. *J. Agric. Sci. Finl.* 56: 193-198.
- , **Suvitie, M. & Seppälä, J.** 1978. Timoteinurmen sato, koostumus ja sulavuus kasvukauden eri aikoina (in Finnish). *Kehittyvä Maatalous* 39: 27-35.
- Syrjälä-Qvist, L., Pekkarinen, E., Setälä, J. & Kangasmäki, T.** 1984. Effect of nitrogen fertilization on the protein quality of timothy grass and silage. *J. Agric. Sci. Finl.* 56: 193-198.
- Terry, R.A. & Tilley, J.M.A.** 1964. The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an *in vitro* procedure. *J. Br. Grassl. Soc.* 19: 363-372.
- Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J.** 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.* 48: 185-197.
- Thomas, C.** 1980. Conserved forages. In: *Feeding Strategies for Dairy Cows*. pp. 8.1-8.14. Ed. W.H. Broster, C.L. Johnson & J.C. Tayler. Agricultural Research Council, London.
- , 1984. Milk compositional quality and the rôle of forages. In: *Milk compositional quality and its importance in future markets*. Ed. M.E. Castle and R.G. Gunn. Occ. Publ. No. 9, BSAP. pp. 69-76.
- , 1987. Factors affecting substitution rates in dairy cows on silage based rations. In: *Recent Advances in Animal Nutrition* (ed. W. Haresign and D. J. A. Cole). Butterworths, London. pp. 205-218.
- , **Daley, S.R., Aston, K. & Hughes, P.M.** 1981. Milk production from silage. 2. The influence of the digestibility of silage made from the primary growth of perennial ryegrass. *Anim. Prod.* 33: 7-13.
- & **Thomas, P.C.** 1988. Factors affecting the nutritive value of grass silages. In: *Recent Developments in Ruminant Nutrition* (ed. W. Haresign and D. J. A. Cole). Butterworths, London. pp. 274-307.
- Thomas, P.C., Chamberlain, D.G., Robertson, S., Shamoan, S.A. & Watson, J.N.** 1984. The effect of the source and level of protein supplement on milk production in dairy cows given silage diets. *Proc. 7th Silage Conference, Belfast.* pp. 45-46.
- , **Chamberlain, D.G., Kelly, N.C. & Wait, M.K.** 1980a. The nutritive value of silages. Digestion of nitrogenous constituents in sheep receiving diets of grass silage or grass silage and barley. *Br. J. Nutr.* 43: 469-479.
- , **Kelly, N.C., Chamberlain, D.G. & Wait, M.K.** 1980b. The nutritive value of silages. Digestion of organic matter, gross energy and carbohydrate constituents in the rumen and intestines of sheep receiving diets of grass silage or grass silage and barley. *Br. J. Nutr.* 43: 481-489.
- Thorvaldsson, G. & Andersson, S.** 1986. Variations in timothy dry matter yield and nutritional value as affected by harvest date, nitrogen fertilization, year and location in Northern Sweden. *Acta Agric. Scand.* 36: 367-385.
- & **Fagerberg, B.** 1988. Effects of weather on nutritional value and phenological development of timothy. *Swedish J. Agric. Res.* 18: 51-59.
- Tilley, J.M.A. & Terry, R.A.** 1963. A two stage technique for the *in vitro* digestion of forage crop. *J. Br. Grassl. Soc.* 18: 104-111.
- Tuori, M., Kaustell, K. & Huhtanen, P.** 1998. Comparison of the protein evaluation systems of feed for dairy cows. *Livest. Prod. Sci.* 55: 33-46.

- , **Kaustell, K., Valaja, J., Aimonen, E., Saarisalo, E. and Huhtanen, P.** 2000. Rehutaulukot ja ruokintasuositukset [in Finnish, Feed tables and feeding recommendations]. Yliopistopaino, Helsinki, Finland. 3rd ed. 88 p.
- Vanhatalo, A., Dakowski, P. & Huhtanen, P.** 1996. Effects of stage of growth and duration of rumen incubation time on intestinal digestibility of rumen-undegradable nitrogen of grass by mobile-bag method in cows. *Acta Agric. Scand. Section A Animal Sci.* 46: 1-10.
- & **Toivonen, V.** 1993. Microbial protein synthesis in cattle fed silage cut from grass fertilized with different levels of N. *Proc. 10th International Silage Conference*, Dublin, Ireland. pp. 168-169.
- Van Soest, P.J.** 1967. Development of a comprehensive system of feed analyses and its application to forages. *J. Anim. Sci.* 26: 119-128
- . 1994. *Nutritional ecology of the ruminant*. 2nd ed. Cornell University Press, Ithaca, NY. 476 p.
- , **Mertens, D.J. & Deinum, B.** 1978. Preharvest factors influencing quality of conserved forage. *J. Anim. Sci.* 47: 712-720.
- van Vuuren, A.M., Huhtanen, P. & J.-P. Dulphy.** 1995. Improving the feeding and health value of ensiled forages. In: M. Journet, E. Grenet, M.-H. Farce, M. Thériez, C. Demarquilly (eds), *Recent developments in the nutrition of herbivores. Proc. IVth Int. Symp. on the Nutrition of Herbivores*. INRA Editions, Paris. pp. 279-307.
- Waldo, D.R., Smith, L.W. & Cox, E.L.** 1972. Model of cellulose disappearance from the rumen. *J. Dairy Sci.* 55: 125-129.
- Weston, R.H.** 1996. Some aspects of constraint to forage consumption by ruminants. *Aust. J. Agric. Res.* 47: 175-197.
- Williams, B.A., Oostdam, A.J., Groot, J.C.J., Boer, H. & Tamminga, S.** 2000. Effects of ageing on the *in vitro* fermentation of cell walls and cell contents of entire, fractionated and composite leaves of Italian ryegrass. *J. Sci. Food Agric.* 80: 484-490.
- Wilman, D. & Adesogan, A.** 2000. A comparison of filter bag methods with conventional tube methods of determining the *in vitro* digestibility of forages. *Anim. Feed Sci. Technol.* 82: 33-47.
- Zinn, R.A. & Owens, F.N.** 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* 66: 157-166.
- Åman, P. & Lindgren, E.** 1983. Chemical composition and *in vitro* degradability of individual chemical constituents of six Swedish grasses harvested at different stages of maturity. *Swedish J. Agric. Res.* 13: 221-227.